POLLEN PHYSIOLOGY OF ALKALOID YIELDING SOLANUMS (S. KHASIANUM CLARKE AND S. MARGINATUM L. f.) AND FLOWER BUD DEVELOPMENT OF S. KHASIANUM IN VITRO

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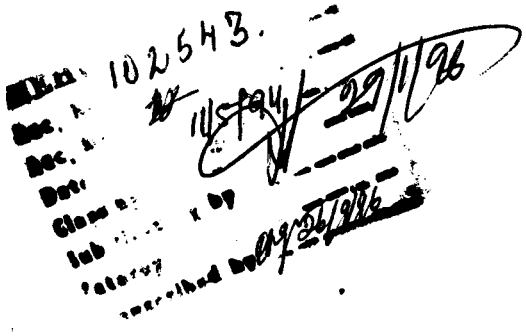

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I certify that the thesis entitled **Pollen Physiology of alkaloid yielding Solanums (S. khasianum Clarke and S. marginatum L.f.) and Flower bud development of S. khasianum in vitro**, submitted by Mr. Vituo Belho for the Degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by him under my supervision. The thesis presented is worthy of being considered for the award of the Ph.D. Degree. This work has not been submitted for any Degree of any other University.

SHILLONG
The 6. 3, 1992

Y. S. Chauhan
SUPERVISOR

Dedicated
to
My Father, Mother
and
All My Teachers

C O N T E N T S

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Chapter-I

GENERAL INTRODUCTION

The social and economic importance of steroidal drugs were emphasized by Bradley et al. (1979). Steroidal hormones comprise nearly 6% of present pharmaceutical preparations and are the active ingredients in drugs used for the cure of addison's disease, rheumatoid arthritis, leukaemia, chronic cases of asthma and obesity. These hormones are also the active principle of oral contraceptive.

Diosgenin, a steroid sapogenin of Dioscorea is used commercially for the synthesis of steroidal drugs. Due to paucity of Dioscorea raw material availability attempts were made to find out alternate sources of raw material

for steroid industry. Like diosgenin, alkaloid solasodine a nitrogenous analogue of diosgenin obtained from the berries of Solanum species, also yields pregnadienolene acetate, a key intermediate for the synthesis of steroidal drugs (Sato et al., 1951). For the above reason, solasodine yielding solanums have received increasing attention as a convenient and most promising alternate source of raw material for the commercial synthesis of hormonal steroids (Schreiber, 1979). Solasodine occurs as glycosides in more than 100 species of Solanum (Schreiber, 1968). Both Solanum khasianum and S. marginatum yield solasodine in commercially exploitable amount (Chaudhuri and Rao, 1964; Saini et al., 1965; Chaudhuri and Hazarika, 1966; Maiti et al., 1964, 1965; Cruz and Proana, 1970; Chauhan and Joshee, 1985). Solanum khasianum, native to India, is widely distributed in the sub-continent while S. marginatum, a native of Ethiopia introduced in Meghalaya (India) by Chauhan and Joshee (1985), has economic potential as a source of solasodine (Cruz and Proano, 1970). Berries of S. marginatum contain solasodine much higher than any other Indian species (Chauhan and Joshee, 1985). Among the solanums indigenous to India S. khasianum berries contain the highest percentage of solasodine (Kaul and Zutshi, 1977).

The commercial cultivation of these species, however, is hampered due to difficulty in harvesting of

berries because of presence of sharp spines on the aerial parts of the plant, asynchronous flowering resulting in unsynchronized berry maturity and low berry yield due to andromonoecy. Therefore, the need to genetically improve S. khasianum has been emphasized, but attempts to improve S. khasianum by conventional hybridization methods have not succeeded (Kaul and Zutshi, 1974). Thus there is a need to try alternate methods for genetic improvement of solasodine yielding species of Solanum. Indeed mutations are considered an alternative to hybridization and recombinations in plant breeding. Using mutagens attempts were made to synthesize new types of S. khasianum rich in solasodine content, less spiny and high berry yielder (Matsunaga et al., 1969; Bhatt, 1975, 1977). However, attempts have not met with much success.

Ionizing radiations are increasingly used to induce mutations in plants and many medicinal and aromatic plants have been improved by inducing mutations with gamma-rays (Kapoor and Datta, 1967). Gamma rays have been tried for the genetic improvement of S. khasianum and although Bhatt (1972) could get a mutant having curved and blunt spines success has not been spectacular (Bhatt, 1972; Chauhan et al., 1975a,b, 1976; Ravindran, 1981).

Stair and Mergen (1964) suggested that pollen irradiated with low level of radiation could be used to

obtain mutations. Further, the mutants thus obtained are rarely chimeric (Briggs, 1970). Pfahler (1983) emphasized that gametophytic selection is more effective than sporophytic selection in plant breeding. However, for successful crop improvement programme a thorough understanding of the radiobiology of the species being improved is essential since great differences (more than 100 fold) exist in the radiosensitivity of different species (Sparrow et al., 1961a,b). Rudolph (1971) considers that information on relative radiosensitivity of different species under comparable conditions is of value and helps in understanding the radiobiological responses of the species. However, for the success of plant breeding programme employing radiated pollen the first pre-requisite is to understand pollen physiology and pollen radiobiology of the species concerned. Investigations of in vivo pollen germination are not easily feasible due to involvement of complex pistillate tissue. Therefore, in vitro studies are used for investigating physiology of pollen germination and pollen tube growth. Since information on these aspects are lacking for pollen of S. khasianum and S. marginatum an attempt was made to investigate these aspects in S. khasianum and S. marginatum.

Stylar heteromorphism, that is, the occurrence of flowers with different syle length in the same inflorescence of an individual is common in many species of Solanum (Hossain, 1973). Depending on the style length, two types of flowers can be recognised, viz. (i) long-styled flowers which are female fertile and bear fruit, and (ii) short-styled flowers which are female sterile flowers and do not bear fruit. Such stylar variation is also found in S. khasianum (Murty and Abraham, 1975). Several attempts were made to find out the factors responsible for stylar heteromorphism in solanums. The variations in style size has been attributed to weather and season (Pal and Singh, 1943), nutrition (Wakhloo, 1972; Hossain, 1973). In S. khasianum occurrence of long- and short-styled flowers could be altered by exogenous application of growth hormones (Chauhan and Ravindran, 1980; Chauhan and Joshee, 1987). Using tissue culture techniques De Jong and Bruinsma (1974b,c) found the involvement of growth hormones in the development of pistil in Cleome flowers. Thus in vitro flower bud culture studies can be used as a tool to investigate hormonal involvement in flower developement. Although the flower buds of some plant species have been cultured successfully, the potentiality of the flower buds for growth, development, and differentiation in in vitro

experiments varies from species to species (Konar and Kitchlue, 1982). In a number of studies young flower buds of diverse species have been cultured *in vitro* (Konar and Kitchlue, 1982). However, the success in obtaining normal growth of buds to maturity in both dicotyledons (Konar and Nataraja, 1964; Mohan Ram and Wadhi, 1966; Ganapathy, 1969) and monocotyledons (Guha and Johri, 1966) was limited. Tepfer *et al*. (1963, 1966) used complex medium for the growth of young floral buds of *Aquilegia* and although initiation and early development of various floral organs was achieved, the petals did not grow to maturity and stamen primordia aborted in later stages. In *Viscaria*, the floral apices with sepal or petal primordia could be reared to maturity only if a pair of leaves was included with the floral buds (Blake, 1966, 1969). Only in a few cases the normal development of stamens and ovules and the differentiation of pollen grains (Black, 1966, 1969; Porath and Galun, 1967; Hicks and Sussex, 1970;Polowick and Greyson, 1982) and embryo sac (Hicks and Sussex, 1970) has been achieved. In *Lycopersicon esculentum*, however, normal development of young floral buds occurred *in vitro* (Rastogi and Sawhney, 1986, 1988). In all these studies nutritional and hormonal requirements vary from species to species. But no attempt has been made so far to culture young floral buds of *S. khasianum*

to determine its nutritional and hormonal requirements. Therefore, an attempt is made to investigate these aspects.

Thus it is evident from the above that not much work has been done on physiology of pollen germination and pollen radiobiology of S. khasianum and S. marginatum and in vitro flower bud development of S. khasianum. Therefore, in the present study an attempt was made to investigate the following aspects of pollen physiology, radiobiology and in vitro flower bud culture :

I. Pollen germination :
   - A. Nutritional requirement
   - B. Effect of growth hormones
   - C. Radiosensitivity
   - D. Modulation of radiation response
   - E. Cytochemistry.

II. Fertilizing ability of irradiated pollen.

III. M_1 generation of S. khasianum.

IV. Flower bud development of S. khasianum in vitro.

Chapter-II

REVIEW OF LITERATURE

Research Materials

Family Solanaceae is of immense economic value because solanaceous plants are used as vegetable, medicines and ornamentals. Lately, solanums have recieved much attention due to the discovery of glycoside-solasodine which yields pregnadienolene acetate an intermediate compound for the synthesis of steroid hormones (Sato et al., 1951; Mann, 1978). Amongst the 31 Indian solanums investigated for the presence of solasodine, Solanum khasianum is the most promising species for the production of steroid hormones (Chaudhuri and Rao, 1964; Saini et al., 1965; Maiti et al., 1964,

1965). Solanum marginatum, an exotic species introduced in Shillong, also yields solasodine in commercially exploitable amount (Schreiber, 1968; Cruz and Proano, 1970). The solasodine content of the berries of S. marginatum is much higher compared to any Indian species (Chauhan and Joshee, 1985).

Large scale cultivation of many alkaloid yielding solanums has met with limited success due to low berry yield resulting in low alkaloid recovery. The low berry yield of these species is due to the occurrence of andromonoecy which is manifested in the occurrence of heterostylous morphic styles (Hossain, 1973; Murty and Abraham, 1973; Dulberger et al., 1981). In S. khasianum the frequency of long-styled female fertile (fruit bearing) flowers ranges between 30-40% only while rest of the flowers are short-styled female sterile (non-fruit bearing) (Kaul and Zutshi, 1977). In S. marginatum only 8.14% of flowers produce fruits (Dulberger et al., 1981).

Attempts to hybridize S. khasianum with other species of solanums have not been successful (Kaul and Zutshi, 1974; Pearce and Lester, 1979; Rao, 1979); likewise crosses between S. marginatum and S. melongena did not succeed (Pearce and Lester, 1979). Efforts were also made to evolve mutant types of S. khasianum having

less spines and high solasodine content, using mutagens/colchicine (Matsunaga et al., 1969; Janaki Ammal and Bhatt, 1971; Bhatt, 1972, 1975, 1977; Bhatt and Heble, 1978). However, these efforts met with limited success. The use of two mutagens, like radiation and colchicine to obtain desirable mutants has been emphasized by Bhatt and Heble (1978). Gamma radiation had been tried by irradiating the seeds of S. khasianum, however, the results were not spectacular (Ravindran, 1981). Similarly, Chauhan et al. (1975a,b, 1976) although could obtain considerable variation in berry yield in R_2 generation by using gamma radiation on seeds of S. khasianum, solasodine content did not change appreciably.

POLLEN PHYSIOLOGY

Since the observation of pollen germination and tube growth by Von Mohl (1834) many studies have been done on pollen germination, pollen tube growth and the factors influencing pollen germination and tube elongation. Many reviews on pollen physiology, structure and chemistry have appeared since then (Johri and Vasil, 1961; Linskens, 1964; Rosen, 1968; Vasil, 1974; Mascarenhas, 1975; Johri et al., 1977; Shivanna et al., 1979; Johri and Shivanna, 1977, 1985; Heslop-Harrison, 1987). Pollen of some plants

may germinate easily under a wide range of conditions while in others the requirements may be very exacting, failing which there may be no germination (Johri and Vasil, 1961). The following factors have a bearing on pollen germination and pollen tube elongation :

Carbohydrate

Normally pollen grains do not germinate satisfactorily in water, but aqueous solutions of sucrose (occasionally other sugars), with or without addition of accessory substances, induce good germination (Vasil, 1960). The requirement of an external source of carbohydrate for pollen germination has been demonstrated using carbon labelled sugar (Hrabetova and Tupy, 1964). Several sugars and sugar derivatives such as sucrose, glucose, fructose, dextrose, galactose, raffinose, mannose, mannitol and rhamnose have been tested (Bishop, 1949; Faull, 1955; O'Kelly, 1955; Raghavan and Baruah, 1956; Vasil, 1960; Hrabetova and Tupy, 1961, 1963, 1964). Although other sugars also support good germination and tube growth, sucrose is a better source for pollen germination and pollen tube growth in most of the plant species investigated (Tupy, 1960; Hrabetova and Tupy, 1964; Cook and Walden, 1965; Patil and Rahman, 1978; Nakamura, 1978). Hrabetova and Tupy (1964) stated that in

49 species belonging to 24 families including Solanaceae sucrose has higher growth effect than its components. They found that the rate of ^{14}C incorporation from sucrose into lipids and polysaccharides was much more intense than the carbon from other sugars. The better tube growth of apple pollen on sucrose medium was attributed to the higher respiration rate (Hrabetova and Tupy, 1961). Specific growth effects of sucrose on pllen tube elongation are due to the presence of bound β-D-fructofuranose (Hrabetova and Tupy, 1961). It is considered that sucrose is exogeneously hydrolysed by the invertase released by pollen, and only hydrolysed products are taken up by pollen (Dickinson, 1967; Nakamura *et al.*, 1980). However, biochemical studies conducted by Dehusses *et al.* (1981) indicated that the uptake of unhydrolysed sucrose is linked with inward movement of protons.

The sucrose concentration required for optimal pollen germination varies from species to species. The two-celled pollen require 10-20% sucrose while three-celled pollen require even higher concentrations of sucrose often upto 50% (Shivanna and Johri, 1985). Whereas in *Solanum aviculare*, *S. laciniatum*, *S. indicum* and *S. khasianum* 15% sucrose in the medium supported maximum pollen germination and pollen tube growth

(Ravindran and Chauhan, 1980), S. sisymbrifolium pollen require only 10% sucrose for optimum germination and tube elongation (Kuruvilla et al., in press). Similarly, the optimum sucrose concentration for pollen germination and pollen tube growth in Eucalyptus globulus, E. morrisbyi, E. ovata and E. urnigera also differ markedly with very little germination occurring in the absence of sucrose (Potts and Marsden-Smedley, 1989). Pine pollen though do not require exogenous carbohydrate to germinate in vitro (Hellmers and Machlis, 1956) exogenous supply of sucrose (0.03-0.1%) enhances pollen germination and tube elongation in Pinus kesiya (Katiyar and Chauhan, 1988).

Boron

Essentiality of boron for pollen germination and pollen tube growth was first reported by Schmucker (1935). Incorporation of boron in the germinating medium not only improved pollen germination and tube growth but also optimised pollen germination and tube elongation over a wide range of sugar concentrations in many plant species (De Bruyn, 1966; Portnoi and Horovitz, 1977). The role of boron in pollen germination and pollen tube growth has been extensively reviewed by Johri and Vasil (1961), Dnyansagar (1974), Shivanna et al. (1979) and Shivanna and Johri (1985). The optimal boron requirement for pollen

germination and tube elongation differs in different species : Graminae (150 ppm), Cucurbitaceae (200 ppm) and Leguminosae (100-150 ppm) (Vasil, 1964). Dnyansagar (1974) working on members of Leguminosae obtained similar results. Vasil (1958, 1960) investigated the effects of various concentrations of boric acid on pollen germination and pollen tube growth in a number of crop plants and reported that whereas 100-150 ppm boron favoured germination and tube elongation the higher concentrations induced inhibition in most cases.

Pollen of Sesbania aegyptica, however, requires only 50 ppm of boron for optimum germination and tube growth (Dnyansagar, 1974). Crotolaria juncea pollen though a member of Leguminosae does not require boron for optimal germination and tube elongation (Vasil, 1964). Pinus kesiya pollen although do not require boron for germination and tube growth incorporation of 5 ppm boron in the germinating medium optimises germination and tube elongation (Katiyar and Chauhan, 1988). Pollen of certain plant species can tolerate very high concentrations of boron (1200 ppm) which otherwise induce inhibition in other pant species (Visser, 1955). Boric acid is a better source of boron than borax for pollen germination and pollen tube growth (Vasil, 1964).

Sugar-agar/gelatin medium is though suitable for the germination of various species of Solanum (Johri and Vasil, 1961) certain Solanaceae members require boron (150 ppm) also for pollen germination and tube elongation (Vasil, 1964). Solanum aviculare, S. laciniatum, S. indicum and S. khasianum pollen require 100-200 ppm of boric acid for optimum germination and tube elongation (Ravindran and Chauhan, 1980). S. sisymbrifolium pollen require 100 ppm of boric acid for optimal germination and tube elongation (Kuruvilla et al., in press).

Boron influences many physiological and biochemical processes in plants, e.g. translocation and metabolism of carbohydrates (Gauch and Dugger, 1953; Dugger et al., 1957; Stanley and Loewus, 1964; Young et al., 1966; Chen and Loewus, 1977) and growth hormones/regulators (Coke and Whittington, 1968; Rajaratnam and Lowry, 1974; Bohnsack and Albert, 1977; Smirnov et al., 1977). Boron deficiency results in accumulation of toxic concentration of IAA (Coke and Whittington, 1968). However, Smirnov et al. (1977) considers that boron deficient symptoms are not due to the accumulation of IAA, but due to the accumulation of oxidised phenols. De Bruyn (1966) suggested that boron counteracts the toxic effects of IAA. McLeod (1975), however, could not find such effect in tomato pollen.

Vasil (1964) concluded that the stimulatory effects of boron in pollen germination and tube growth might be due to (a) an increased absorption, translocation and metabolism of sugars by forming sugar-borate complexes, (b) increased oxygen uptake, and (c) its role in the synthesis of pectic materials for pollen tube growth.

Calcium

Brink (1924) showed that pollen grains in larger populations during culture germinated better than when lesser number of pollen grains are cultured. This phenomenon was termed 'population effect' or 'mutual stimulation effect' (Brewbaker and Kwack, 1963). Brewbaker and Majumder (1961) reported that the population effect factor was heat stable, water soluble, and highly diffusable substance. Subsequently, Brewbaker and Kwack (1963) identified this factor to be calcium ion. They demonstrated that calcium could replace pollen growth factors. Brewbaker and Kwack (1963) concluded ... the pollen population effect to be essentially universal in its occurrence. Hence they formulated a germinating medium having 300 ppm of calcium. This medium has been widely used ever since. However, pollen grains of many taxa do not require exogenous supply of calcium for germination and tube growth (Glenk et al., 1969). This may be due to

the presence of higher levels of endogenous calcium in the pollen of these taxa or due to contamination of the culture medium (Shivanna and Johri, 1985). In Juglans nigra calcium could not overcome population effect (Hall and Farmer, 1971).

Calcium gives rigidity to the pollen tube wall by binding to pectic carboxyl groups (Kwack, 1967). Dickinson (1967) emphasized the role of calcium in controlling the permeability of pollen tube membrane. Calcium plays an important role in the growth of pollen tube tip (Weisenseel and Jaffe, 1976; Reiss and Herth, 1979; Picton and Steer, 1983; Polito, 1983a,b). In Tradescantia virginiana calcium is involved in vesicle fusion as well as the plasticity of the tip wall components of pollen tube (Picton and Steer, 1982, 1983). Besides, calcium may be involved in protein phosphorylation (Polya et al., 1986) and sucrose synthetase enzyme activity (Nakamura, 1978).

Magnesium

Certain ions, including magnesium when added in the culture medium enhance the stimulatory effect of calcium (Brewbaker and Kwack, 1963). In absence of these ions calcium was not active in overcoming population

effect. Since then magnesium has been routinely used for pollen of many plant species including Carex ovalis and C. nigra (Shivanna and Heslop-Harrison, 1981), Plumeria alba (Shivanna, 1977; Johri and Shivanna, 1977). However, pollen of some plant species do not require an exogenous supply of magnesium for pollen germination (Cook and Walden, 1965; Pfahler, 1968; Pfahler and Linskens, 1973; Hoekstra, 1973; Shivanna et al., 1978; Shivanna and Heslop-Harrison, 1981).

Potassium

Besides magnesium, the other supporting ions which enhance the stimulatory effect of calcium are potassium and sodium (Brewbaker and Kwack, 1963). Brewbaker and Kwack (1963) observed that only 34% of Ornithogalum virens pollen could germinate in presence of sucrose, boron, calcium and magnesium but when potassium was incorporated in the medium pollen germination was enhanced to 50.5%. Further, optimum pollen germination occurred in the presence of both magnesium and potassium as supporting ions for the calcium effect. Pollen of Eleocharis palustris (Shivanna and Heslop-Harrison, 1981) and Plumeria alba (Shivanna, 1977; Johri and Shivanna, 1977) also required potassium for better germination and tube growth. On the otherhand, pollen of many plant species do

not require potassium (Pfahler, 1965; Glenk et al., 1969; Rao and Ong, 1972; Ferrari and Wallace, 1975; Roberts et al., 1983) for pollen germination.

Brewbaker and Kwack (1963) stated that pollen tubes do not require exogenous potassium or magnesium for growth. According to them, these cations may maximise the association of calcium in the cell wall, which in turn leads to structural rigidity and physiological properties such as permeability and ion selectivity to the wall.

Temperature

Pollen of most plants show optimum germination and tube growth between 20°-30°C (Johri and Vasil, 1961). The correlation between temperature and pollen germination is usually represented by an optimum curve (Roberts and Struckmeyer, 1948; Visser, 1955). At low temperature the percentage of germination and tube growth are considerably reduced (Smith and Cochran, 1935). Pollen of Pinus kesiya do not germinate at 15°C while optimum germination and maximum tube elongation occurs at 25°C and 30°C respectively (Katiyar and Chauhan, 1988). In Antirrhinum pollen while negligible pollen tube growth occurred at 15°C, optimum germination and maximum tube elongation occurred at 25°C (Smith, 1942). In Bryophyllum pollen

also though appreciable germination occurred at 15°C, optimum germination and tube growth was evident only at 25°C (Smith, 1942). In Solanum aviculare and S. laciniatum 91.2% and 75% of pollen germinated at 15°C respectively (Ravindran and Chauhan, 1980). In these species whereas varying temperatures (15-30°C) did not have any appreciable effect on pollen germination, the pollen tube growth was greatly affected by temperature; optimum tube elongation occurred at 25°C (Ravindran and Chauhan, 1980). In S. indicum and S. khasianum, optimum pollen germination and tube elongation occurred at 30°C; lower temperatures (15°-25°C), however, induced inhibition (Ravindran and Chauhan, 1980). S. sisymbrifolium pollen require 25°C for optimum pollen germination and tube growth. Lower and higher temperatures were inhibitory (Kuruvilla et al., in press). Optimum germination of red pepper pollen occurs at 35°C (Hirose, 1957). Similary, germination of Rosa hugonis pollen occurs at 28°-35°C (Koncalova, 1975).

Increasing temperature have enhancing effect on the diameter of the pollen tubes (Smith, 1942). Smith (1942) found that pollen tubes of Antirrhinum showed pronounced broadening and 'bloating' (Swelling) of the distal portion of tube at 30°C and extensive bursting of the bloated tubes at 35°C. He further mentioned that at 35°C a great

diversity of pollen tube lengths resulted with many short, broad tubes and some extremely long ones with broadened proximal portions. Vasil and Bose (1959) also observed marked swelling or bursting of the tips at higher temperature.

pH

Investigations on the effect of pH on pollen germination and pollen tube growth are limited to only few taxa (Shivanna and Johri, 1985). Pollen grains germinate in a wide range of pH, but best results are obtained at pH 5.5-6.5 (Johri and Vasil, 1961). Although, pollen grains of each taxon apparently have an optimal pH range, slight deviations seldom have drastic effect (Shivanna and Johri, 1985).

In Malus (Speranza and Calzoni, 1980) and Crotolaria (Sharma and Shivanna, 1983) satisfactory pollen germination occurs at pH 4-9. In species of Pinus, pollen germination and tube elongation are satisfactory between pH 4.5-7.3 (Tanaka, 1955; Nygaard, 1969; Katiyar and Chauhan, 1988). However, the frequency of long pollen tubes in Pinus kesiya was more at pH 6-7.3 (Katiyar and Chauhan, 1988). In Lathyrus odoratus optimum germination occurs at pH 7.0 and the pH range favourable for pollen germination is rather narrow (Brink, 1925). Pollen of

Vinca rosea show maximum germination and pollen tube elongation at pH 6.5 (Bandyopadhya and Mukerjee, 1977). Sharma and Shivanna (1983) have shown that pollen diffusates have the ability to shift the pH of the germinating medium to its optimum requirement in acidic and alkaline conditions, hence pollen are able to germinate in a wide range of pH.

Johri and Vasil (1961) stated that pH may not directly affect pollen germination but whatever effect is produced may be entirely due to the cations and anions present in the medium in the form of buffer salts. Therefore, they suggested that instead of using different buffer mixtures, NaOH-HCl may be used for adjusting the pH of the medium.

Gelling

Pollen germination and tube growth are generally divided into four phases : imbibition or hydration phase, lag phase, tube initiation phase and rapid tube elongation phase (Linskens and Kroh, 1970).

Recent studies reveal that hydration phase is critical for the subsequent processes of pollen germination and tube elongation (Shivanna and Johri, 1985). Controlled hydration seems to be more important for successful germination and tube growth (Hoekstra and

Bruinsma, 1975; Shivanna and Heslop-Harrison, 1981; Shivanna et al., 1981). Many reports of better germination on agar or gelatin medium, compared to liquid medium, have been interpreted as the result of controlled hydration of the pollen. Addition of agar to the culture medium has been shown to have enhanced pollen tube growth, although it could not increase germination (Brewbaker and Kwack, 1963).

Growth hormones

Pollen grains are rich in hormones hence they generally do not require exogenous supply of these substances for germination and tube growth (Lunden, 1956; Stanley and Linskens, 1974). However, in many plant taxa pollen germination and pollen tube growth are influenced by exogenous application of growth hormones. But the effects are highly variable.

Cytokinins, auxins and gibberellins induced stimulation (Raghavan and Baruah, 1956, 1959; Chandler, 1957; Konar, 1958; Bose, 1959; Datta and Chaudhury, 1965; Rosen, 1968; Shukla and Tewari, 1973; Malik and Chhabra, 1976; Wee and Rao, 1979; Malasi et al., 1989) and inhibition (De Bruyn, 1966; Tsukamoto and Matsubara, 1968; Taylor, 1972; Sondheimer and Linskens, 1974; Mehan and Malik, 1975; Ravindran and Chauhan, 1986) of pollen

germination and pollen tube growth are reported in a number of plant species. However, pollen of different species vary in their responses to growth hormones. The responses of pollen are also influenced by the particular growth hormone and concentration used. For example, IAA, gibberellin and zeatin do not affect in vitro germination and tube growth of Petunia hybrida pollen (Sondheimer and Linskens, 1974) but NAA and GA_3 inhibit pollen germination and pollen tube growth in this species (Virk and Grover, 1978). McLeod (1975) found tomato pollen almost completely insensitive to auxins and gibberellins. IAA suppressed pollen tube growth in Calotropis but GA_3 stimulated pollen tube elongation in this species (Mehan and Malik, 1975). Both IAA and GA_3 stimulate pollen germination in Arachis (Malik and Chhabra, 1976). In Ananas GA_3 and NAA significantly increased tube length but not germination, while IAA, 2,4-D and kinetin had no effect either on germination or tube elongation (Wee and Rao, 1979). Pollen of Solanum khasianum and S. indicum have different response to IAA, kinetin and GA_3 treatments (Ravindran and Chauhan, 1986). In S. indicum low concentrations of GA_3 stimulated pollen tube elongation while higher concentrations induced inhibition. All the concentrations of IAA and kinetin, however, induced inhibition. Unlike S. indicum, in S. khasianum all the three hormones caused inhibition (Ravindran and

Chauhan, 1986). Kuruvilla et al. (in press) found that in S. sisymbrifolium low concentrations of GA_3 and kinetin promoted pollen germination and tube growth but higher concentrations were inhibitory.

Studies pertaining to the effect of ethylene on pollen germination and pollen tube elongation are limited and contradictory (Shivanna and Johri, 1985). In peach ethylene stimulated pollen germination (Buchanan and Briggs, 1969) while in others (Cajanus, Crotolaria, Impatiens, Trigonella, Zephyranthes, S. khasianum, S. indicum) it was inhibitory (Sastri, 1974; Chauhan and Kharbteng, 1986). Still in some other species (Arachis hypogaea) ethylene though suppressed pollen germination it promoted tube elongation (Malik and Chhabra, 1976). Abscisic acid though stimulates and inhibits pollen germination and pollen tube elongation in many plant species (Shukla and Tewari, 1973; Sondheimer and Linskens, 1974; Malik and Singh, 1975; McLeod, 1975; Mehan and Malik, 1975; Malik and Chhabra, 1976; Barlow et al., 1961) pollen of some plant species (Pinus mungo) are not influenced by ABA (Nygaard, 1969).

In tomato pollen low concentration of IAA when applied at 1, 2 and 3 hr after culture failed to influence germination and tube elongation rate (McLeod, 1975). But at higher concentrations it suppressed germination at

these time intervals (McLeod, 1975). However, when high concentrations of IAA were added after 3 hr culture, there was a pronounced stimulatory effect on tube growth. At higher concentrations several other growth inhibitors also inhibited germination to various extents, but promoted tube elongation. This led McLeod (1975) to suggest that germination and tube elongation are two distinct processes which differ in their sensitivity to different chemicals.

POLLEN RADIOBIOLOGY

Radiosensitivity

Pollen sensitivity to ionizing radiations varies from species to species (Brewbaker and Emery, 1962). Brewbaker and Emery (1962) observed that the estimated doses reducing pollen germination by 50% in 18 plant species ranged between 35-550 krad while LD_{100} for two species (Buddleia asiatica, Lilium longiflorum) were found between 600 and 700 krad. Visser and Oost (1981) observed that LD_{50} for germination and tube elongation of apple and pear pollen was about 220 krad. In different Cucumis species studied by Denissen and Den Nijs (1987) LD_{50} for pollen germination and tube growth was estimated at 380 krad. In contrast, maize pollen has an average LD_{50} dose of 54.1 krad and total lethal dose of 120 krad (Pfahler, 1971).

Most of the investigations dealing with the effect of ionizing radiation on pollen germination and tube growth reveal a linear relationship between exposure dosage and pollen germination and tube elongation (Brewbaker and Emery, 1962; Pfahler, 1971, 1973; Gilissen, 1978; Visser and Oost, 1981; Denissen and Den Nijs, 1987) thus pollen germination and pollen tube growth decrease with the increasing radiation dose.

Radiation-induced stimulation of pollen germination and pollen tube growth has been reported in several plant species, Pinus silvestris (Zelles and Seibold, 1976), P. wallichiana and P. patula (Katiyar and Chauhan, 1987a,b), Nicotiana tabacum and N. rustica (Swaminathan and Murthy, 1959), Pseudotsuga menziesii (Livingston and Stettler, 1973). Katiyar and Chauhan (1987b) observed that the percentage occurrence of polysiphony; a common phenomenon in P. patula, decreased with the increasing radiation dosage.

Different tissues and cell types of the same plant differ in their radiosensitivity (Nilan, 1956; Bacq and Alexander, 1959). Meiotic cells are generally more sensitive to ionizing radiations than somatic cells (Sax and Swanson, 1941; Sparrow and Singleton, 1953). The most important factors determining the sensitivity of sporophytic tissue to ionizing radiations are nuclear

volume (Sparrow and Evans, 1961; Sparrow et al., 1961a,b, 1963) and interphase chromosome volume (Sparrow et al., 1963, 1968; Yamakawa and Sparrow, 1965). Polyploids of Chrysanthemum and Sedum exhibit increasing resistance with the increasing degree of polyploidy suggesting that increasing chromosome number without changing nuclear volume has a protective effect (Sparrow et al., 1961a,b). The single most important parameter for radiosensitivity is thus interphase chromosome volume, having an inverse relationship with radiosensitivity (Sparrow et al., 1963; Yamakawa and Sparrow, 1966). In pollen this relationship is less distinct (Pfahler, 1971). Similarly, Brewbaker and Emery (1962) could not find any correlation between the volume of generative nuclei and the radiosensitivity. However, they advocated that a correlation between the pollen size and radiosensitivity exists; larger grains being more sensitive to ionizing radiations than smaller grains. Pfahler (1971) also supported this view. According to Brewbaker and Emery (1962) although ionizations per unit volume remain constant for pollen of differing volumes, the probability that cellular poisons such as peroxides mediate much of the damage may serve as one rational for the apparent proportionality of size and radiosensitivity. An exceptional case against this hypothesis is found in Lilium longiflorum pollen. In comparison with other pollen grains, L. longiflorum pollen

is large but LD_{50} value is also comparatively high (Brewbaker and Emery, 1962). According to Denissen and Den Nijs (1987) sensitivity of the pollen in respect to pollen germination is not dependent on the volume of interphase chromosome or DNA content. Resistance of pollen tube growth against irradiation is, however, explained by variation in the amount of DNA per nucleus; the species with higher amount of DNA being more resistant.

Ionizing radiations-induced injury causes bursting of pollen during germination decreasing germination percentage (Pfahler, 1971, 1973; Gilissen, 1978). In maize pollen, percent of ruptured pollen increased with the increasing gamma exposure (Pfahler, 1971). Gilissen (1978) reported that x-ray irradiated pollen showed normal swelling soon after placement in germination medium but these ruptured after sometime pressing out part of the protoplasm. Apparently, the cell membrane is affected in such a manner that rupturing of pollen grains occurs before germination (Pfahler, 1971). Cresti et al. (1977) also observed that in the stylar intercellular spaces, degenerated pollen tube materials were present due to the radiation-induced bursting of pollen tubes in Lycopersicum peruvianum.

The primary effect of irradiation on pollen grains is considered to be associated with the structure and/or the synthesis of the cell membrane (Pfahler, 1971, 1973; Gilissen, 1978). Gilissen (1978) further stated that radiation-induced rupturing of Petunia pollen in germinating medium occurred due to an alteration in plasma membrane fluidity caused by oxidation of unsaturated compounds. Pai and Gaur (1987) suggested that gamma rays induced increase in the cholesterol : unsaturated fatty acid ratio increases fluidity of the membrane. Radiation also adversely affects phospholipids by destroying lipid moiety (Pai and Gaur, 1982). The high lethal doses and sigmoidal nature of radiation-killing curves for pollen tube growth suggest a cumulative physiological action, affecting principally the cell membrane and cellular machinery involved in synthesis of cellulose, pectin and callose (Brewbaker and Emery, 1962).

Ultrastructural aspects of pollen tube growth inhibition after gamma irradiation were investigated by Cresti et al. (1977) and Speranza et al. (1982). In apple, pollen tubes developed from gamma irradiated pollen failed to attain precise zonation and organisation (Speranza et al., 1982). Similarly, in Lycopersicum peruvianum, pollen tubes were destroyed by a precise degradation process which led to the disappearance of the inner wall, lysis of the pollen tube, accumulation of

several bipartite particles and alteration of endoplasmic reticulum into a whorl of concentric rings (Cresti et al., 1977).

Physiological and Biochemical Effects

Besides membrane damages, ionizing radiations also influence physiological and biochemical processes in plant cells. Gunckel and Sparrow (1961) stated that radiation effect on some vital physiological processes could be mediated through a modification of enzyme activity, or change in the effective level of a substrate, or a growth-promoting/inhibiting substance. They observed that most enzymes so far investigated revealed a change in activity at suitable doses but there was no consistent pattern with regard to the response. Amongst the enzymes studied the activity of amylase, cellulase, ribonuclease, peroxidase, invertase, and acid phosphatase was stimulated (Stone and Cherry, 1972; Calzoni and Speranza, 1982; Bagi et al., 1988) while the activity of mitochondrial adenosine triphosphatase, inorganic pyrophosphatase, lactate dehydrogenase, alcohol dehydrogenase, As-D-estarase and β-glucosidase was significantly reduced (Khanna and Maherchandani, 1980; Georgieva and Atanassov, 1986; Pai and Gaur, 1987). High doses of gamma irradiation either did not affect or slightly decreased the activity of glutamate, isocitrate, malate, glucose-6-phosphate

dehydrogenases, a-esterase and acid phosphatase in pollen tubes of Lilium regale and Beta vulgaris (Georgiéva and Atanassov, 1986). Similarly, Calzoni and Speranza (1982) could not detect any siginificant change in the activity of alkaline phosphatase, peroxidase and glycan-hydrolases in gamma irradiated apple pollen. The enhanced activity of peroxidase by gamma irradiation in sweet potato discs has been correlated with enhanced production of ethylene (Ogawa and Uritani, 1970). Georgiéva and Atanassov (1986) suggested that radioresistance of dehydrogenases, a-estarase and acid phosphatase could be responsible for the high resistance of pollen.

Gamma radiation influences utilization of sugars by plant tissue. Inoue et al. (1975a,b, 1980) observed that in rice seeds gamma radiation reduced the participation of the pentose phosphate pathway in glucose catabolism. In Lilium and Beta pollen, high doses of gamma radiation inhibited activity of lactate dehydrogenase and alcohol dehydrogenase which take part in the anaerobic breakdown of carbohydrates as well as As-D-esterase (pectin estarase) and β-glucosidase which are involved in the cell wall metabolism (Georgiéva and Atanassov, 1986).

Radiation inhibits protein synthesis in plant tissues (Gunckel and Sparrow, 1961). Radiation-induced inhibition of protein synthesis was demonstrated by a decreased rate

of incorporation of firmly bound deuterium and ^{14}C labelled glycine (Kuzin, 1956a,b). Inhibition of protein synthesis in irradiated plants was associated with the inhibition of oxidative phosphorylation, the source of energy for the synthetic process (Gunckel and Sparrow, 1961). Such possible uncoupling action of gamma radiation in oxidative phosphorylation in excised bean hypocotyl segments was also demonstrated by Joshi and Gaur (1970). However, Calzoni and Speranza (1982) observed that more protein were released from irradiated pollen (600 krad) in apple. Van der Donk et al. (1978) found that polyribosomes in X-ray irradiated Douglas fir pollen were more active in protein synthesis. They suggested that the enhanncing effect of irradiation was due to its influence on de- and re-masking of mRNA.

Radiation also inhibits both RNA and DNA synthesis (Gunckel and Sparrow, 1961; Dunham et al., 1971). Inhibition of RNA synthesis in irradiated plant material could be due to an increase in RNase which preferentially destroys the mRNA in polysomes (Bagi et al., 1988). Yealy and Stone (1975) suggested that in lettuce seeds gamma irradiation selectively inhibited ribosomal RNA synthesis delaying seed germination. The connection repeatedly observed between radiation effect and changes in genetic structure has led to the common opinion that damage or

changes of DNA are the first initiated radiation effect (Simonis, 1966). Several workers have reported high sensitivity of DNA synthesis to ionizing radiations (Gunckel and Sparrow, 1961). The effects of ionizing radiations of DNA synthesis has been critically reviewed by Gunckel and Sparrow (1961). Most of the works reviewed by them indicated that DNA synthesis was inhibited by ionizing radiation. Reduced content of nucleotides has been reported in irradiated barley seeds (Bjornseth et al., 1957).

Conflicting reports have appeared in literature concerning the effect of ionizing radiations on phytohormones, particularly auxins. Gordon (1956) claimed that the reduction of auxin supply in plants was brought about by inactivation of an auxin synthesizing system, especially the enzyme which converts indole-acetaldehyde to indole-acetic acid. However, Miura et al. (1974) could not observe much suppression of this enzyme system. Raj Gopal (1971) working with the same enzyme system observed that X-ray instead of causing reduction of auxin synthesis increased auxin yield. Similarly, Croci et al. (1990) reported that in garlic seed cloves gamma radiation-induced presence of substances with auxin-like activity which corresponded to IAA. Jordan and Haber (1974) conducted studies on cytokinins and mitotic inhibition in gamma

plantlets and concluded that cytokinin was not affected by gamma radiation. However, Pandey *et al*. (1978) observed that gamma irradiation of *Haworthia mirabilis* tissue in callus culture resulted in the formation of cytokinins. They suggested that free cytokinins were produced by a pathway that included synthesis and degradation of cytokinin-containing transfer RNA. Sideris *et al*. (1971) observed that GA_3 in aqueous solution was extremely sensitive to ionizing radiation and the relationship between the dose and GA_3 inactivation was exponential one. However, their attempts to inactivate GA_3 *in situ* using barley seedlings with gamma radiation failed, but in seedlings from irradiated caryopses the concentration of the gibberellin-like substances was reduced.

Gamete Formation

Cytological aberrations due to ionizing radiations have been reported in many plant species (Catecheside, 1948; Sudhakaran, 1972; Sinha and Godward, 1972; Kalloo, 1972; Kumar and Das, 1973; Mujeeb and Greig, 1973; Banerji and Chatterjee, 1988; Das and Roy, 1989; Kumar and Roy, 1989; Kumar and Sinha, 1989). The spectrum of cytological aberrations observed in pollen tube mitosis is essentially the same as that observed at mitotic metaphase in other tissues (Brewbaker and Emery, 1962). The meiotic irregularities observed in pollen mother cells include

irregular pairing, translocation, inversions, stickiness or clumped configuration at metaphase I, bridges with or without fragments and laggards at metaphase I (Sinha and Godward, 1972; Kalloo, 1972; Sudhakaran, 1972; Kumar and Das, 1973; Das and Roy, 1989; Kumar and Sinha, 1989). Sarmah (1989) observed unequal chromosome distribution, laggards and chromatin bridges as a result of gamma irradiation of seeds of Solanum khasianum and S. indicum. All these studies indicated that meiotic irregularities increase with the increasing exposure of ionizing radiation. Ionizing radiations cause aberrations in pollen tube mitosis : chromatid breaks; isochromatid breaks involving both paired chromatids and exchanges (Brewbaker and Emery, 1962). In irradiated apple pollen the generative cell and the vegetative nucleus remain inside the grain (Speranza et al., 1982). Vassileva-Dryanovska (1966) observed that in Tradescantia pollen subjected to X-ray or gamma irradiation undivided or abortively divided generative nuclei combined with fragments were common at 10-100 krad. X-ray irradiated generative nuclei rarely divide in Nicotiana (Brewbaker and Emery, 1962; Grant et al., 1980).

Fertilizing Ability

Ionizing radiations have pronounced effect on the reproductive capacity of many plant species (Brewbaker and

Emery, 1962; Stairs, 1964; Stairs and Troendle, 1969; Clausen, 1973a; Van Den Boom and Den Nijs, 1983). Pollen of some plants exposed to 500-600 krad of ionizing radiations were able to germinate and produce pollen tubes in vitro (Vassileva-Dryanovska, 1966; Georgieva and Atanassov, 1986). In Lycopersicum pimpinellifolium pollen tubes reached ovules even after exposure to 100 krad of X-ray (Nishiyama and Uematsu, 1967). In Tradescantia pollen exposed to 500 krad of X-ray and gamma rays were capable of germination and produced pollen tubes which could penetrate embryosac (Vassileva-Dryanovska, 1966). Brown and Cave (1954) found that 4 krad of X-ray did not alter the germinability and fertilizing ability of Lilium formosanum pollen. Gamma irradiation upto 5 krad though did not alter in vitro germination and tube growth, as well as fertilizing ability of maize pollen it drastically reduced kernel set (Pfahler, 1967). Uematsu and Nishiyama (1967) observed that in Lycopersicum pimpinellifolium fruit set was not affected but fruit weight and seed set were reduced in proportion to radiation exposure given to pollen. Percentage of normal fruit and number of seeds per fruit in Lycopersicon esculentum (Constant et al., 1971), Cucumber (Van Den Boom and Den Nijs, 1983) and Nicotiana rustica (Caligari et al., 1981) were reduced as a reult of pollen irradiation. Increasing exposure of radiation decreased number of filled seeds and viability

of the filled seeds in Betula nigra (Clausen, 1973a). Several studies also indicated that treatments of pollen with ionizing radiations resulted in the development of parthenocarpic fruits (Kgazal, 1989; Sharma and Hacq, 1989).

In Tradescantia Vassileva-Dryanovska (1966) observed three types of nuclear fusion when pollen was exposed to various doses of X- and gamma radiation : (a) normal fusion as in untreated material, (b) nuclear fusion took place but the normal changes in the female nuclei chromatin did not occur and most of these nuclei did not divide, and (c) nuclear fusion did not occur but the male chromatin was appressed to the female nucleus which might be stimulated to start division.

Effects on F_1 Progenies

The foremost visible effect of radiation in both irradiated seed and seeds developed due to irradiated pollen is the inhibition of seed germinability (Brewbaker and Emery, 1962; Clausen, 1973b; Ravindran, 1981; Kgazal, 1989). Abnormalities of cotyledon also increased with the increasing radiation (Devreux and Mugnozza, 1964).

The growth and development of plant is inversely proportional to the dose rate or total dose of ionizing radiation administered (Gunckel, 1965). Radiation-induced

inhibition of growth occurs in cereal seedlings (Duanovic and Ehrenberg, 1965), Corn (Woodstock and Combs, 1965), Pinus species (Mergen and Thielges, 1966; Katiyar, 1991), wheat, sorghum and radish (Woodstock and Justice, 1967), Sequoia gigantea (Taylor, 1968), safflower (Chauhan, 1969) and Betula nigra (Clausen, 1973b). Sax (1963) discussed the stimulatory effects of ionizing radiations on plants. However, Sheppard and Hawkins (1990) strongly opined that the stimulation of plant growth by very low doses of ionizing radiations; 'radiation hormesis', is an elusive phenomenon since it is not reproducible. Radiation inhibited axillary buds formed localized swelling with wide bases on the stem and short shoots of hybrids of Nicotiana (Meiselman et al., 1961).

Almost all plants produce leaf abnormalities following exposure to suitable doses of ionizing radiations. Radiation caused leaf abnormalities (change in colour-chlorosis, form and texture) have been reported in safflower (Chauhan, 1969).

Due to ionizing radiations flowering plants often display reduced size, abscissed buds and aborted flowers (Brewbaker and Emery, 1962). Abnormal flowers developed in irradiated Solanum khasianum and S. indicum (Ravindran, 1981). Radiation-induced pollen sterility has been reported in many plants (Brewbaker and Emery, 1962; Kumar

and Sinha, 1989). The significance and utility of pollen sterility as an index of radiation genetic damage is emphasised by Brewbaker and Emery (1962) while reviewing the works done on morphological effects occurring in progenies of irradiated pollen.

Modulation of Radiation Responses

The responses to ionizing radiations can be modified by several post-irradiation treatments (Klein and Klein, 1971). These treatments can result in a decrease or increase of the severity of the injury (Latarjet and Gray, 1954). Phytohormones occupy an important place among the various treatments and agents that can modulate radiation injury.

Post-irradiation treatments of GA_3 modulates the growth responses of radiation-stunted maize seedlings (Haber and Luipold, 1960; El-Keredy et al., 1975), and pinto bean plants (Lockhart, 1961). Mathur (1961, 1965) using GA_3 could reverse gamma-ray-induced potato dormancy. Bhattacharya (1977) observed that GA_3 was superior to IAA in reversing the inhibitory effects of higher exposures of gamma radiation in soyabean plants. Silveira and Hell (1977) stated that post-irradiation GA_3 treatments reduced the severity of radiation-induced growth inhibition in Phaseolus vulgaris plantlets.

Romani (1966) suggested that auxins might have a significant role in correcting inhibitory effects of gamma-irradiation. Holmsen et al. (1964) using auxin reported reversal of radiation-inhibited geotropic response in corm and pea seedlings. Similarly, followed by GA_3, IAA was the most effective agent in modifying gamma radiation-induced sprouting inhibition in garlic seed cloves; BAP being the most effective agent (Croci et al., 1987). When rice seeds exposed to gamma rays were treated with IAA there was a significant recovery in growth (Bhattacharya and Rao, 1978). Abrol et al. (1969) observed that pre soaking of wheat in IAA solution could protect α-amylase activity against gamma rays induced inhibition. Singh (1984) found that pretreatments with growth hormones (IAA and GA_3) were effective in decreasing the magnitude of x-ray induced physiological injury in Hordeum vulgare but post-treatments were found to be more effective in enhancing the efficiency of radiation.

Araratyan et al. (1975) studied radio-protective effects of kinetin on chromosome aberrations induced by x-ray irradiation of Crepis capillaris seeds. They found that kinetin stimulated the onset of synthetic stage for certain portion of cells. Mikhailov et al. (1978) found that post irradiation soaking of pea seeds in kinetin solution could partially protect the seedling growth and

development from inhibitory effects of x-ray. However, kinetin failed to restore/induce cell division inhibited by gamma radiation in wheat seedlings (Jordan and Haber, 1974). In S. khasianum, GA_3 and IAA could not modulate radiation-induced inhibition of long-styled flowers, but application of kinetin resulted in significant recovery from gamma radiation caused injury (Chauhan and Ravindran, 1980). Low concentrations of GA_3, IAA and kinetin could improve pollen germination while higher concentrations of kinetin further reduced germination of pollen in Solanum indicum plants developed from irradiated seeds (Ravindran, 1981). In S. khasianum, all these hormones at low concentrations remained ineffective while higher concentrations further reduced pollen germination in seed irradiated plants (Ravindran, 1981). Modulatory effects of growth hormones on germination and tube growth (stimulation/inhibition) of irradiated pollen through their effect on cytochemical constituents in pine pollen have been investigated by Katiyar (1991).

FLOWER BUD CULTURE

In vitro floral bud culture was first attempted by La Rue (1942). However, it was two decades later that Galun et al. (1962, 1963) achieved success in growing detached flower buds of Cucumis sativus. They could modify sex

expression by manipulating growth hormones in the culture medium. Subsequent to this report, Tepfer et al. (1963, 1966) grew the flower buds of Aquilegia to maturity. Aquilegia was used by Bilderback (1971, 1972) to study the effect of amino acids and growth hormones on floral bud development in vitro. Though the addition of growth hormones (IAA, GA_3 and kinetin) enhanced bud development, complete differentiation of floral primordia into mature organs was not achieved. Blake (1966) for the first time reported complete development of the excised buds into flowers. Excised floral apices of two species of Viscaria (V. candida and V. cardinalis) were raised on MS medium (Murashige and Skoog, 1962) supplemented with coconut milk, casein hydrolysate, amino acids, GA_3 and IAA. However, the development of the flower to maturity occurred rarely unless the explants had a pair of leaves. Blake (1969) made a detailed study of the factors affecting the development of floral parts in V. candida. Floral buds of 1-2 mm diameter were cultured to assess the production of pollen. Unlike other parts, production of pollen bearing anthers was greatly affected by constituents of the medium and environmental condition like temperature. Development of ovary and ovules tended to be irregular and consistent effects of treatment could not be obtained (Blake, 1969).

Floral primordia of Nicotiana tabacum had been successfully cultured by Hicks and Sussex (1970). Buds with only sepal primordia were cultured on Linsmaier and Skoog's (1965) medium with 0.01-10 ppm kinetin. The petal, stamen and carpel primordia formed in normal acropetal sequence in cultured buds. Buds grown on 0.01 and 0.1 ppm of kinetin though revealed uni- and/or bi-nucleate pollen grains in anthers, these did not germinate successfully. In vitro grown floral buds though formed well developed ovaries comparable to those found in nature fertilization with normal pollen did not succeed (Hicks and Sussex, 1970). Similarly, Rastogi and Sawhney (1986) cultured young floral buds of tomato with sepal primordia only. The cultured buds showed normal flower development through to anthesis and a full complement of floral organs was produced. In the anthers, microsporogenesis occurred and microspores and pollen grains were formed. The gynoecium differentiated ovary contained many ovules. Rastogi and Sawhney (1988) also cultured a male sterile stamenless mutant of tomato in medium supplemented with BAP and GA_3. Well developed flowers with a normal complement of floral organs were produced in medium containing BAP and GA_3.

Inflorescence of Begonia franconis comprising two male flowers (1-2.5 mm) and one female (0.4-0.7 mm) were

reared on artificial medium to study the requirements for optimum growth (Berghoef and Bruinsma, 1979). They found that for satisfactory growth, both nitrate and ammonium along with a cytokinin were essential. The optimum concentration of cytokinin required for female bud was much higher than that for male bud.

In nature, the inflorescences of Cleome exhibit alternation of fruited (fertile) and sterile zone of pistil development (De Jong and Bruinsma, 1974a). Flower buds of Cleome iberidella (about 2.5 mm long) were cultured in vitro to study the effect of nutrients and growth hormones on the development of floral parts (De Jong and Bruinsma, 1974b; De Jong et al., 1974). Petal growth was more sensitive to carbohydrate and nitrogen deficiencies than pistil growth. Pistil development was favoured by low pH levels (De Jong et al., 1974). Pistil development in flower buds of C. iberidella grown in vitro was strongly stimulated by specific cytokinin, viz. zeatin and BAP; kinetin was less active while 2-ip was ineffective (De Jong and Bruinsma, 1974b). In presence of zeatin low concentrations of NAA stimulated pistil development, whereas higher concentrations were inhibitory. Gibberellins inhibited pistil development and caused female abortion in both C. iberidella and C. spinosa (De Jong and Bruinsma, 1974a,b). In vitro culture

of excised floral buds has been reviewed extensively by Konar and Kitchlue (1982).

Chapter-III

POLLEN GERMINATION

INTRODUCTION

The pollen grains represent a highly reduced male gametophyte of higher plants and are produced in large quantity in the anthers. During their free life stage, they have the unique property of behaving like independent organisms. When pollen land on compatible receptive stigma, they produce pollen tubes which grow through style and reach embryosac in the ovule. Within the pollen tube two male gametes are formed which are released once the pollen tube enters the ovule leading to double fertilization in angiosperms. Thus factors controlling pollen germination and pollen tube elongation are

important in the life cycle of seed plants and their understanding is essential in the applied aspects of plant breeding programmes.

Stigma provides a suitable site for pollen germination. However, pollen germination studies *in vivo* are not easily feasible because of the complex pistillate tissue system. Nevertheless, it is possible to germinate pollen grains in a simple medium and achieve a reasonable length of pollen tube growth. Information about physiological factors influencing pollen germination and pollen tube growth has largely been obtained using *in vitro* studies (Shivanna and Johri, 1985).

Pollen germination requirements vary from species to species (Shivanna and Johri, 1985). Apart from moisture they require a carbohydrate source, boron, calcium, magnesium and potassium. Optimal conditions for pollen germination and tube elongation have been worked out only in a few taxa (Shivanna and Johri, 1985). Besides, temperature, pH and gelling of the medium also affect pollen germination and pollen tube growth (Johri and Vasil, 1961). Therefore,an attempt was made in the present study to understand the physical and nutritional requirements of *in vitro* pollen germination and tube growth of *Solanum khasianum* and *S. marginatum*. For the purpose, temperature, pH, gelling, sucrose, boron,

calcium, magnesium and potassium requirements of S. marginatum pollen were investigated in the present study. Further, as the effects of temperature, sucrose and boron on pollen germination and pollen tube growth of S. khasianum have already been studied (Ravindran and Chauhan, 1980), only pH, gelling, calcium, magnesium and potassium requirements of S. khasianum pollen were investigated in this study.

Growth hormones affect pollen germination and pollen tube growth (Johri et al., 1977). However, the response of pollen to various concentrations of growth hormones varies from species to species (Johri and Vasil, 1961). In the present study an attempt was made to investigate the effect of kinetin, IAA and GA_3 on germination and tube growth of both S. khasianum and S. marginatum pollen.

In crop improvement programme it is sometimes required to irradiate the material under the conditions of maximum and minimum radiosensitivity (Gunckel and Sparrow, 1961). Although great differences exist in the radiosensitivity of various species (Sparrow et al., 1961a,b) still information on radiosensitivity of different species under comparable conditions is of significance (Rudolph, 1971).

Brewbaker and Emery (1962) stated that pollen grains of flowering plant are excellent experimental material for

radio-biological investigations. These are unicellular structures having haploid condition with lesser number of variable than the seeds. Thus these are relatively simple target for low penetrating radiations. In addition the use of pollen for the induction and study of mutations has many commendable features. Stair and Mergen (1964) have also suggested that pollen irradiated with low level of radiation could be used to obtain mutation. Pfahler (1983) emphasized that the gametophytic selection is more effective than sporophytic selection in plant breeding. A great advantage of the pollen irradiation method as opposed to the seed irradiation for growing plants is the fact that the former rarely produce chimeras (Briggs, 1970).

Van der Donk et al. (1978) reported that x-irradiation of pollen in douglas-fir interferes with the main regulatory mechanism of pollen tube growth. A decrease in the rate of both DNA and RNA, and protein synthesis occurs following irradiation with moderate doses of radiation (Setlow and Pollard, 1962). It is also presumed that certain enzymes present in small number but responsible for certain vital syntheses, such as vitamin and hormone production could be lost due to irradiation (Luse, 1970).

Ravindran (1981) investigated the effect of gamma radiation on seed germination, seedling survival, growth, yield, shoot apical meristem, flowering responses and heterostyly in S. khasianum and S. indicum. He also investigated pollen germination and pollen tube growth in both the species collected from plants raised from irradiated seeds. However, the direct effect of gamma radiation on pollen germination and pollen tube growth and cytochemistry of irradiated pollen in S. khasianum and S. marginatum have not been investigated so far. Therefore, in the present study, radiation effects on the following aspects of S. khasianum and S. marginatum pollen have been investigated :

1. Effect of gamma rays doses on pollen germination and pollen tube growth.
2. Modulation of radiation responses of irradiated pollen with growth hormones (Kinetin, Indole acetic acid and Gibberellic acid).
3. Cytochemistry (carbohydrates, protein and nucleic acids-RNA) of control and irradiated pollen treated with/without growth hormones.

MATERIAL AND METHODS

Pollen grains of S. khasianum and S. marginatum were collected from plants raised in the experimental garden of the Department of Botany, NEHU, Shillong. For pollen collection and inoculation just anthesized flowers were brought to the laboratory and the following procedures

were followed for the two species :

S. khasianum

1. Pollen Collection and Inoculation

Anthers were collected from just anthesized flowers and the tips were cut off with a sharp scalpel as described by Ravindran and Chauhan (1980). The pollen were then dusted on pre-cooled and semi-solid basal medium for pollen germination by gently tapping the anther.

2. Basal Medium

Modified Brewbaker and Kwack (1963) basal medium for in vitro study of pollen germination and pollen tube growth was used to investigate pollen germination and tube elongation in S. khasianum. The medium comprised of 15% sucrose, 200 ppm boric acid (Ravindran and Chauhan, 1980), 300 ppm calcium nitrate, 100 ppm potassium nitrate and 200 ppm magnesium sulphate (Brewbaker and Kwack, 1963). The pH of the medium was adjusted to 7.3 and 0.6% bacto-agar was incorporated into the medium for gelling.

A drop of the above semi-solid medium was placed on clean coverslips, allowed to cool and then dusted with pollen grains. Subsequently, pollen dusted coverslips were inverted and placed over a metallic ring prefixed to a glass slide and sealed with petroleum jelly to prevent drying of the medium and for maintaining moisture inside

the metallic cavity during incubation. Pollen inoculated slides were incubated at 30°C; the optimum temperature for the germination of S. khasianum pollen (Ravindran and Chauhan, 1980).

3. pH

The effect of acidity on pollen germination and pollen tube growth of S. khasianum were investigated by altering pH of the basal medium mentioned above. The pH of the medium was adjusted to 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5. The pollen inoculated slides were incubated for 2 hrs. Having determined the pH optima (6.5) for pollen germination and tube elongation, in all the subsequent experiments, the pH of the medium was adjusted to 6.5.

4. Nutrients

Calcium, magnesium and potassium influence pollen germination and tube elongation (Brewbaker and Kwack, 1963). Therefore, concentration of calcium, magnesium and potassium required for optimising germination and tube growth in S. khasianum pollen were investigated by incorporating different concentrations of calcium nitrate (0, 1, 5, 10, 50, 100, 200, 300 and 400 ppm), magnesium sulphate (0, 100, 200, 300 and 400 ppm) and potassium nitrate (0, 100, 200, 300 and 400 ppm) in the basal medium.

5. Gelling

Effect of gelling on pollen germination and pollen tube growth was investigated by incorporating different concentrations of bacto-agar in the medium. For this purpose, 0, 0.2, 0.6, 1.0, 1.4 and 1.8% concentrations of agar were incorporated in the medium.

S. marginatum

1. Pollen Collection and Inoculation

Pollen grains of S. marginatum were collected in a clean petriplate by gently tapping the anthers. Approximately, 7-10 mg of pollen could be obtained from every flower. Collected pollen grains were dusted, as in case of S. khasianum, on coverslips having a drop of semi-solid basal medium. The collected pollen grains were spread over the medium by dipping a fine camel hair brush in the pollen and gently tapping it over the coverslips having pre-cooled medium. The incoulated coverslips were inverted over metallic rings prefixed on glass-slides and then sealed with petroleum jelly as in case of S. khasianum.

2. Basal Medium

The basal medium containing 10% sucrose, 100 ppm boric acid, 300 ppm calcium nitrate, 200 ppm magnesium sulphate, 100 ppm potassium nitrate and 0.6% bacto-agar

(Brewbaker and Kwack, 1963) was used for germinating S. marginatum pollen. The pH of the medium was adjusted to 7.3. Using above basal medium effects of various factors for pollen germination and pollen tube elongation were investigated.

3. Temperature

The effects of varying temperature on pollen germination and pollen tube growth of S. marginatum were studied first using the aforementioned basal medium. For this purpose, inoculated slides were incubated at 20, 25, 30 and 35°C for 2 hrs.

Having determined the optimum temperature (30°C) for the species, in subsequent experiments, slides were incubated at 30°C for 2 hrs.

4. pH

The effects of acidity on pollen germination and pollen tube growth of S. marginatum were investigated by altering the pH of the basal medium. The pH used were 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5. Having determined the pH optima (6.5) of germination and tube elongation, in subsequent experiments, pH of the medium was always adjusted to 6.5.

5. Nutrients

The optimal requirement of sucrose for S. marginatum pollen is not known. Therefore, optimum sucrose requirement of germinating S. marginatum pollen were found out by incorporating different concentrations of sucrose (0, 10, 15 and 20%) in the basal medium.

Different concentrations of boric acid (0, 100, 200, 300 and 400 ppm) were incorporated to find out optimum concentration of boron required for germination of pollen and pollen tube elongation in S. marginatum. Similarly, the optimal concentrations of calcium, magnesium and potassium required for germination and elongation of tube of S. marginatum pollen were determined by incorporating different concentrations of calcium nitrate (0, 100, 200, 300 and 400 ppm), magnesium sulphate (0, 100, 200, 300 and 400 ppm) and potassium nitrate (0, 100, 200, 300 and 400 ppm) respectively in the medium.

Pollen Germination Medium

Based on the above experiments a modified Brewbaker and Kwack's medium was devised for both S. khasianum and S. marginatum considering optimal nutrient requirements of their pollen. A comparative study was also undertaken to compare the performances of different media containing simple sucrose-agar, Brewbaker and Kwack's medium (1963)

and Brewbaker and Kwack's medium modified in view of the present findings. Temperature (30°C), pH (6.5), sucrose (15%) and agar (1%) being the optimum for both the species, were maintained for all the three media in both the species. The composition of the three media tested for the two species are given in **Table 1**.

Table 1: Composition of Pollen Germination Media

Medium	Nutrients (ppm)					
	Sucrose	Boron	Calcium	Magnesium	Potassium	Agar
S. khasianum						
1. Sucrose-agar (control)	15%	0	0	0	0	1%
2. Brewbaker & Kwack's	15%	100	300	200	100	1%
3. Modified Brewbaker and Kwack's	15%	200	10	0	0	1%
S. marginatum						
1. Sucrose-agar (control)	15%	0	0	0	0	1%
2. Brewbaker & Kwack's	15%	100	300	200	100	1%
3. Modified Brewbaker and Kwarck's	15%	200	0	200	200	1%

Growth Hormones

The medium used for investigating effects of growth hormones on pollen germination and pollen tube growth of both the species were the media formulated from the above studies and contained 15% sucrose, 200 ppm boric acid and 10 ppm calcium nitrate for *S. khasianum* pollen and 15%

sucrose, 200 ppm boric acid, 200 ppm magnesium sulphate and 200 ppm potassium nitrate for S. marginatum pollen. In the medium, for both the species 1% agar was added and the pH of the media were adjusted to 6.5.

The growth hormones investigated were 6-furfurylaminopurine (Kinetin), Indole-3-acetic acid (IAA) and Gibberellic acid (GA_3). The concentrations used were 0, 1, 5, 10 and 25 ppm. Kinetin was first dissolved in a 1N NaOH solution. Different concentrations of these growth hormones were incorporated in the modified Brewbaker and Kwack's media for the two species.

Collected pollen grains were dusted, and incubated as in the previous experiments, on a coverslip having a drop of medium. The medium devoid of growth hormone served as control.

Radiation

Anthesized flowers of S. khasianum and S. marginatum were collected in a clean beaker and exposed to radiation in a gamma chamer-900 having ^{60}Co as radioactive source, emitting gamma rays at the rate of 66.95 krad/sec. Gamma radiation at 1, 50, 100, 200, 500, 600, 700 and 800 krad were used in the present study to investigate the effects of radiation on in vitro pollen germination and pollen tube growth in both the species.

Pollen Collection and Incubation

The methods of pollen collection and incubation described above were followed in the present experiments as well. Unirradiated pollen were used as control.

Medium and Incubation

The medium used to investigate the effects of gamma radiation on pollen germination and pollen tube elongation in both the species was the medium devised above for the two species being investigated and described as modified Brewbaker and Kwack's medium **(Table 1)**. The medium lacked in growth hormones.

The pH of the medium, for both the species, was adjusted to 6.5. Pollen dusted slides were incubated at 30°C.

Modulation of Radiation Response

Anthesized flowers of both the species were exposed with the respective LD_{50} doses of gamma rays (S. khasianum 600 krad; S. marginatum 500 krad). Both irradiated and unirradiated pollen of both the species were germinated at 30°C in medium devoid of/supplemented with growth hormones. Kinetin, IAA and GA_3 at 1, 5, 10 and 25 ppm concentrations were incorporated into the medium to investigate their modulatory effects on germination and tube elongation of irradiated pollen in both the species.

Recording of Data and Analysis

The germinating pollen grains of both S. khasianum and S. marginatum, in all the above studies, were fixed at the end of 2 hr incubation period with a drop of F.A.A. (5 ml formaldehyde + 5 ml glacial acetic acid + 90 ml of 30% ethanol). Per treatment 5 replicate slides were maintained. Atleast 500 pollen grains were counted from 5 random microscopic fields per slide. For pollen tube growth at least 30 pollen tubes per treatment per slide selected randomly were measured using ocular micrometer. Thus altogether 150 pollen tubes were measured per treatment and mean elongation determined. The pollen tubes were also classified in groups, based on their size, to determine the effect of various treatments on the frequency occurrence of tubes of varying sizes.

Significance between means was tested with the help of student's 't' test using angular transformation values. Percentage of inhibition/stimulation over control was calculated using the formula :

$$\frac{\text{Treated - Control}}{\text{Control}} \times 100$$

Pollen Cytochemistry

In the present study qualitative cytochemical investigations were also conducted to examine the effects

of gamma radiation and growth hormones either alone or in combination on total carbohydrates, protein and nucleic acids in germinating pollen.

Irradiated and unirradiated pollen of both S. khasianum and S. marginatum were germinated in their respective modified liquid medium by hanging drop method. In this medium all the components remained same as mentioned before except agar which was deleted from the medium. Pollen grains of both the species were irradiated with respective LD_{50} dose of gamma rays. Irradiated and unirradiated pollen were germinated in medium devoid of/supplemented with growth hormones. Since 10 ppm concentration of all the three growth hormones (kinetin, IAA and GA_3) influenced pollen germination and pollen tube growth in both the species, effect of this concentration on radiation responses of irradiated pollen were investigated. Germinating pollen were fixed after 2 hr of incubation. Germinating pollen were transferred to a clean slide pre-coated with gelatin adhesive (Jensen, 1962) and fixed with 1-2 drops of neutral 4% formaldehyde. Neutral formaldehyde was prepared by keeping formaldehyde over marble chips for over a month. The slides were air-dried and stained qualitatively for total insoluble polysaccharides protein and nucleic acids using the following methods :

Total Insoluble Polysaccharides

Periodic acid Schiff's (PAS) reaction (Hotchkiss, 1948; McManus, 1948) was used to determine total insoluble polysaccharides. The air dried slides containing pollen were washed in water and placed in 0.5% periodic acid solution in distilled water at room temperature for 40 minutes. These slides were again washed in running water and stained in Schiff's reagent for 15 minutes at 4°C. The slides were rinsed in water and placed in 2% sodium bisulphite for 2 minutes. They were again washed in water, dehydrated through alcohol-xylol series and mounted with D.P.X.

Protein

Mercuric bromophenol blue (Mazia et al., 1953) was used to stain and localise total proteins in the germinating pollen tubes. The tubes fixed with 4% neutral formaldehyde were washed in water and immersed in mercuric bromophenol blue for 15 minutes. These pollen tubes were further rinsed in 0.5% acetic acid for 20 minutes. Next, they were treated with Sorensen's buffer (pH 6.5) for 3 minutes and dehydrated in alcohol-xylol series and mounted in D.P.X.

Nucleic Acids

Nucleic acids were localized using Azure B stain (Flax and Himes, 1952). The slides were stained in

solution of Azure B in citrate buffer at pH 4.00 for 2 hrs at 50°C. The stained slides were washed in water and dehydrated through ethyl alcohol series to xylene and mounted in D.P.X.

The three chemical components tested (total insoluble polysaccharides, protein and nucleic acids) were assessed qualitatively based on their staining intensity. For the purpose of observation and data recording pollen tubes were divided into three regions namely apical, sub-apical and distal region. All the reactions wer evaluated using four grade colour intensity scale :

\+ faint

\++ moderate

\+++ high

\++++ very high.

RESULTS

S. khasianum

Tables 2a and **2b** give data on the effects of pH (5.0-7.5) on pollen germination and pollen tube elongation in S. khasianum **(Fig. 1)**. Pollen germination percentages though did not differ significantly between pH 5.0-6.0, significant reduction in germination percentage occurred

at pH 7.0 and 7.5. Optimum pollen germination (53.26%) occurred at pH 6.5 **(Table 2a, Fig. 2).** Pollen tube elongation increased with the increasing pH upto pH 6.5 and thereafter declined with the increase in pH **(Table 2a, Fig. 2).** Thus maximum mean elongation (226.50 μm) of the pollen tubes was evident at pH 6.5 **(Table 2a, Fig. 2).** **Table 2b** depicts the effect of pH (5.0-7.5) on the frequency of occurrence of pollen tubes of various sizes at a given pH. The smallest pollen tubes measured 15 μm while the longest pollen tubes measured 525 μm **(Table 2b).**

Nutrients :

Calcium

Tables 3a and **3b** give data on the effects of various concentrations (0, 1, 10, 25, 50, 100, 200, 300 and 400 ppm) of calcium on pollen germination and pollen tube elongation, and frequency of occurrence of pollen tubes of varying sizes in S. khasianum. Very low concentration (1 ppm) of calcium did not affect pollen germination. Maximum pollen germination (73.18%) occurred at 10 ppm of calcium **(Table 3a, Fig. 3a).** A decline in the germination of pollen was evident in pollen given calcium concentrations 25 ppm and above but significant inhibition of pollen germination occurred only at calcium concentrations 200-400 ppm **(Table 3a, Fig. 3a).** Elongation of pollen tube increased upto 10 ppm of calcium and thereafter declined **(Table 3a, Fig. 3b).** The

elongation of pollen tube in pollen treated with 1 ppm and 200-400 ppm of calcium though did not differ from control, calcium concentrations 10-100 ppm caused significant elongation of pollen tubes over control **(Table 3a, Fig. 3b).** Maximum pollen tube elongation (304.50 µm) was recorded at 10 ppm calcium **(Table 3a, Fig. 3b).** It is evident from **Table 2b** that lower concentrations (1-25 ppm) of calcium induced more development of long pollen tubes while higher concentrations (50-400 ppm) of calcium inhibited development of long pollen tubes and enhanced development of short pollen tubes **(Table 3b).**

Magnesium

Data on the effects of various magnesium concentrations (100-400 ppm) on pollen germination and pollen tube growth, and pollen tubes of varying sizes are tabulated in **Table 4a** and **4b** respectively. Pollen germination and pollen tube elongation in control medium was 63.72% and 184.50 µm, respectively. All the concentrations of magnesium (100-400 ppm) inhibited pollen germination but only 200-400 ppm could inhibit pollen tube elongation **(Table 4a, Fig. 4).** The intensity of inhibition increased with the increasing concentration of magnesium **(Table 4a, Fig. 4).** Magnesium concentrations favoured development of short pollen tubes in comparison to control **(Table 4b).**

Potassium

Tables 5a and **5b** give data on the effect of various concentrations of potassium on pollen germination and polllen tube elongation in S. khasianum. All the concentrations of potassium (100-400 ppm) inhibited pollen germination and pollen tube elongation in comparison to control **(Table 5a, Fig. 5).** The degree of inhibition increased with the increasing concentrations of potassium **(Table 5a, Fig. 5).** **Table 5b** reveals that percent incidence of long pollen tubes was highest in medium devoid of potassium while incorporation of potassium (100-400 ppm) in the medium reduced incidence of long pollen tubes and enhanced development of short pollen tubes **(Table 5b).**

Gelling

Incorporation of bacto-agar in the germinating medium had significant effects on pollen germination and pollen tube growth in S. khasianum **(Table 6a & b).** Absence of agar in the medium resulted in poor germination of pollen but addition of agar (0.2-1.0%) caused significant improvement in pollen germination **(Table 6a, Fig. 6).** At higher concentrations of agar pollen germination and tube elongation declined **(Table 6a, Fig. 6).** Although pollen germination was significantly inhibited over control when 1.8% agar was used for gelling

of the medium, pollen tubes were significantly bigger than control tubes even at this concentration **(Table 6a, Fig. 6).** Addition of agar in the germinating medium improved the incidence of long pollen tubes and decreased occurrence of short pollen tubes. In control incidence of pollen tubes measuring 15-75 μm was maximum. Incorporation of agar in the medium inhibited development of such short pollen tubes. Pollen germinated on medium having 1% agar developed longer pollen tubes (76-300 μm) in greater frequency and short pollen tubes (15-75 μm) did not form **(Table 6b).** Higher concentrations of agar (1.4-1.8%), however, favoured development of short pollen tubes in comparison to 1% agar **(Table 6b).** But in relation to control the frequency of longer pollen tubes was still more at this agar concentration **(Table 6b).**

Pollen Germination Medium

Table 7a gives data on the effect of three different pollen germinating media on pollen germination and pollen tube growth in S. khasianum. A good amount of pollen were able to germinate (62.47%) in control medium (sucrose 15% + agar 1%) **(Table 7a, Fig. 7).** Brewbaker and Kwack's (1963) medium, however, significantly reduced pollen germination over control **(Table 7a, Fig. 7).** But when modified Brewbaker and Kwack's medium, devised on the basis of pollen germination and pollen tube growth

requirements, worked out above, was used pollen germination was significantly stimulated over control and Brewbaker and Kwack's (1963) medium **(Table 7a, Fig. 7).** Pollen tubes produced by pollen germinated in control medium, at the end of two hours incubation, measured 144.60 µm. Brewbaker and Kwack's (1963) medium significantly increased pollen tube elongation (198.90 µm) over control. But maximum pollen tube growth (266.10 µm) occurred in the modified Brewbaker and Kwack's medium **(Table 7a, Fig. 7).** **Table 7b** gives data on the incidence of varying lengths of pollen tubes produced by pollen germinated in the above media (control, Brewbaker and Kwack's, and modified Brewbaker and Kwack's). In control medium only 3% of pollen tubes attained a size of 301-375 µm. But in Brewbaker and Kwack's (1963), and modified Brewbaker and Kwack's medium their incidence was 24 and 20% respectively **(Table 7b).** Further the longest pollen tubes (376-450 µm) developed by pollen germinated in Brewbaker and Kwack's and modified Brewbaker and Kwack's medium **(Table** 7b). On the other hand, 39% of pollen tubes produced on control medium measured 75-150 µm while in Brewbaker and Kwack's (1963), and modified Brewbaker and Kwack's media the percentage of such pollen tubes was reduced to 11 and 0% respectively suggesting that pollen tube elongation was more pronounced when pollen were germinated on modified Brewbaker and Kwack's medium.

S. marginatum

Temperature

Data on the effects of temperature on pollen germination and pollen tube elongation in S. marginatum **(Fig. 8)** are given in **Table 8a.** Temperature had profound effect on pollen germination and pollen tube growth. At 20°C, 43.55% of pollen germinated and the germination percentage increased with the increasing temperature. Maximum pollen germination (61.67%) occurred at 30°C and thereafter it was drastically reduced to 49.30% at 35°C **(Table 8a, Fig. 9).** High temperature (35°C) also caused bursting of pollen grains. At this temperature 41.79% of pollen bursted within two hours of incubation **(Table 8a, Fig. 9).** Pollen tube elongation in S. marginatum was also greatly influenced by temperature. At 20°C pollen tubes attained a size of 132.90 µm but at 25°C these were 171.90 µm long. Maximum pollen tube elongation (256.20 µm) was evident at 30°C **(Table 8a, Fig. 9).** Pollen tube growth, however, was greatly reduced at 35°C and it measured only 71.10 µm **(Table 8a, Fig. 9).** Further **Table 8b** reveals that incidence of long pollen tubes was more at 25 and 30°C. However, low (20°C) and high (35°C) temperatures favoured development of short pollen tubes **(Table 8b).**

pH

Data recorded on the effects of pH (5.0-7.5) on pollen germination and pollen tube growth in S. marginatum are given in **Table 9a.** At pH 5.0, 44.71% of pollen germinated and germination percentage was enhanced to 53.27% at pH 6.0. Maximum pollen germination (69.16%) was recorded at pH 6.5 **(Table 9a, Fig. 10).** Thereafter, germination percentage declined with the increasing pH and germination percentage was reduced to 50.22% at pH 7.5. Very acidic medium had adverse effect on pollen tube growth in S. marginatum **(Table 9a, Fig. 10).** At pH 5.0 pollen tubes showed an average length of 78.60 µm. Pollen tubes were progressively enhanced with the increasing pH and reached maximum length (178.50 µm) at pH 6.5 **(Table 9a, Fig. 10)** and thereafter, it decreased to 133.20 µm at pH 7.5. However, it remained significantly longer than that at pH 5.0. **Table 8b** gives the data on the effect of various pH (5.0-7.5) on the incidence of pollen tubes of varying length. The incidence of long pollen tubes increased with the increasing pH upto pH 6.5 **(Table 9a, Fig. 10).** The incidence of long pollen tubes declined at pH 7.0-7.5 **(Table 9a).** Maximum percentage of smallest pollen tubes developed at pH 5.0 **(Table 9b).**

Nutrients :

Sucrose

The effects of different concentrations (0-20%) of sucrose on pollen germination and pollen tube growth is

given in **Table 10a.** In control (0% sucrose) only 8.74% of S. marginatum pollen germinated, and this was associated with high percentage (66.92%) of pollen bursting **(Table 10a, Fig. 11).** Addition of sucrose in the medium checked pollen bursting and improved pollen germination **(Table 10a, Fig. 11).** Germination of pollen improved with the increasing concentration of sucrose. Maximum germination (60.71%) of pollen occurred at 15% sucrose. Thereafter a decline in the germination of pollen, became evident. However, germination of pollen was significantly higher in the presence of sucrose than its absence **(Table 10, Fig. 11).** The effects of sucrose concentrations (0-20%) on the incidence of pollen tubes are given in **Table 10b.** In control 90% of the pollen tubes were 15-75 μm long and the remaining 10% measured 76-105 μm. On addition of sucrose in the medium incidence of long pollen tubes improved and the size of pollen tubes increased with the increasing concentration of sucrose in the medium **(Table 10).**

Boron

Table 11a gives data on the effects of various concentrations (100-400 ppm) of boron on pollen germination and pollen tube growth in S. marginatum. All the concentrations of boron (100, 200, 300 and 400 ppm) improved pollen germination. In control pollen

germination percentage was 50.88%. Incorporation of boron concentrations (100, 200, 300 and 400 ppm) in the medium significantly enhanced pollen germination over control **(Table 11a, Fig. 11).** Boron also stimulated pollen tube elongation **(Table 11a, Fig. 12).** In control, pollen tubes were 172.20 µm long; 100 and 200 ppm of boron significantly enhanced pollen tube elongation over control **(Table 11a, Fig. 12).** A decline in the elongation of pollen tubes was evident at higher concentrations (300 and 400 ppm) of boron, however the effect was not significant **(Table 11a, Fig. 12). Table 11b** gives data on the effect of various concentrations (100-400 ppm) of boron on the incidence of pollen tubes of varying length. Occurrence of longer pollen tubes increased with the increasing concentrations of boron upto 200 ppm and thereafter the incidence of long pollen tubes decreased **(Table 11b).**

Calcium

Compared to control, all concentrations (100-400 ppm) of calcium inhibited pollen germination while pollen tube growth was inhibited only by 200-400 ppm **(Table 12a).** The degree of inhibition caused to tube elongation increased with the increasing concentration of calcium (Table 12a, **Fig. 13). Table 12b** gives data on the effect of calcium on pollen tubes of diverse sizes. It is evident from the table that in the absence of calcium percentage of long

pollen tubes was more compared to the tubes developed on media supplemented with 100-400 ppm of calcium **(Table 12b)**.

Magnesium

Data on the effects of different concentrations (100-400 ppm) of magnesium on pollen germination and pollen tube elongation in S. marginatum are given in **Table 13a.** All the concentrations (100-400 ppm) of magnesium stimulated pollen germination and pollen tube growth. The pollen germination and pollen tube elongation increased with the increasing concentration of magnesium upto 200 ppm **(Table 13a, Fig. 14).** Thereafter, the degree of stimulation declined at 300 ppm of magnesium. Least stimulation was observed at 400 ppm of magnesium **(Table 13a, Fig. 14).** **Table 13b** gives data on the effect of magnesium on pollen tubes of diverse sizes. Incorporation of magnesium improved incidence of long pollen tubes in comparison to control **(Table 13b).** The frequency of short pollen tubes was also inhibited by magnesium **(Table 13b).** The incidence of the smallest pollen tubes decreased with the increasing concentration of magnesium upto 300 ppm, but a reverse trend was evident when higher concentration (400 ppm) of magnesium was used **(Table 13b).**

Potassium

Table 14a gives data on the effects of potassium concentrations (100, 200, 300 and 400 ppm) on pollen germination and pollen tube growth in S. marginatum. In control 48.46% pollen germination was observed. Compared to control, germination of pollen was more in potassium treated pollen **(Table 14a, Fig. 15).** However, effect of 100 ppm potassium was not significant **(Table 14a).** The degree of stimulation was maximum at 200 ppm potassium but at higher concentrations (300 and 400 ppm) of potassium the stimulatory effect showed a decline which increased with the increasing concentration of potassium **(Table 14a).** Addition of potassium in the medium, although tended to increase pollen tube elongation it had no statistically significant effect on pollen tube growth **(Table 14a, Fig. 15). Table 14b** shows the effect of potassium on occurrence of pollen tubes of varying sizes. The percentage of longest pollen tubes (226-300 µm) was enhanced from 6% to 12% in 200 ppm of potassium but it declined to 2% at higher concentrations (300 and 400 ppm) of potassium **(Table 14b).** On the other hand, the incidence of short pollen tubes (15-75 µm) was more in control and 100 ppm of potassium. However, the development of short pollen tubes was reduced when 200 and 300 ppm of potassium were added to the medium **(Table 14b).** The frequency of short pollen tubes again increased at higher concentration (400 ppm) of potassium.

Gelling

Data on the effects of gelling on pollen germination and pollen tube growth in S. marginatum is given in **Table 15a.** Although good pollen germination (70.89%) occurred in liquid medium, gelling of the medium with 1% agar significantly improved pollen germination over liquid medium **(Table 15a, Fig. 16).** When other concentrations (0.2, 0.6, 1.4 and 1.8%) of agar were used to gel the medium significant effect on pollen germination was evident **(Table 15a).** Pollen tube elongation also improved as a result of gelling **(Table 15a, Fig. 16).** As compared to control (252.30 µm), addition of 0.6% agar significantly stimulated pollen tube elongation to 273.60 µm. Maximum pollen tube elongation occurred in 1% agar **(Table 15a, Fig. 16).** Higher concentration (1.8%) of agar had inhibitory effect and pollen tube length at this concentration was reduced to 232.50 µm. **Table 15b** gives data on the effect of gelling on the occurrence of pollen tubes of diverse sizes. The frequency of tubes of varying sizes was almost similar to control when 0.2% agar was used for gelling **(Table 15b).** However, percentage of long pollen tubes increased when 0.6 and 1% agar was used **(Table 15b).** Longest pollen tubes (376-450 µm) developed on medium gelled with these concentrations of agar **(Table 15b).** Higher concentrations of agar diminished the development of long pollen tubes **(Table 15b).**

Pollen Germination Medium

Effects of simple sucrose-agar, Brewbaker and Kwack's (1963) and modified Brewbaker and Kwack's medium, as devised on the basis of above studies, on pollen germination and pollen tube growth in S. marginatum are given in **Table 16a.** In simple sucrose-agar (control) medium pollen germination percentage was 49.90%. Brewbaker's and Kwack's (1963) medium supported significantly higher germination (59.46%) but maximum pollen germination (70.49%) occurred in modified Brewbaker and Kwack's medium **(Table 16a, Fig. 17).** Likewise, pollen tube length (258.00 μm) in modified Brewbaker and Kwack's medium was significantly higher while pollen tube length in control and Brewbaker and Kwack's (1963) medium did not differ significantly from each other **(Table 16a, Fig. 17).** **Table 16b** gives data on the effect of the above three media on the incidence of pollen tubes of varying sizes in S. marginatum. The frequency of relatively short pollen tubes (15-150 μm) was more when either control or Brewbaker and Kwack's (1963) medium was used **(Table 16b).** But when pollen were germinated on modified Brewbaker and Kwack's medium the development of long pollen tubes (226-375 μm) was greatly enhanced **(Table 16b).**

Growth Hormones

S. khasianum

Table 17 shows the effects of growth hormones (kinetin, IAA and GA_3) on pollen germination and pollen tube elongation in S. khasianum. In control 84.27% of pollen germinated and pollen tubes attained a size of 300 µm **(Table 17).** It is evident from **Table 17** that while low concentration (1 ppm) of kinetin had no effect on pollen germination and pollen tube elongation, higher concentrations (5, 10 and 25 ppm) inhibited germination **(Table 17, Fig. 18a).** Inhibition of germination and pollen tube elongation over control increased with the increasing concentration of kinetin to the extent that no pollen germinated at 25 ppm of kinetin **(Table 17, Figs. 18a & b).**

IAA

An analysis of IAA effect on pollen germination and pollen tube elongation revealed that 1 ppm of IAA has no effect on pollen germination and elongation of pollen tubes in S. khasianum **(Table 17).** Higher concentrations (5, 10 and 25 ppm) of IAA were, however, detrimental to pollen germination and pollen tube elongation. Pollen germination and pollen tube elongation decreased significantly, in comparison to control, at 5-25 ppm of IAA **(Table 17, Figs. 18a & b).** The degree of inhibition

increased with the increasing concentration of IAA (**Table 17**).

GA_3

Amongst the three growth hormones tested S. khasianum pollen was least sensitive to GA_3. GA_3 at 1, 5 and 10 ppm had no effect on pollen germination and pollen tube growth (**Table 17, Figs. 18a & b**). But 25 ppm of GA_3 inhibited both pollen germination and pollen tube elongation, over control (**Table 17, Figs. 18a & b**).

S. marginatum

Table 18 gives data on the effect of kinetin, IAA and GA_3 on pollen germination and pollen tube elongation in S. marginatum.

Kinetin

All the concentrations (1, 5, 10 and 25 ppm) of kinetin decreased pollen germination and pollen tube elongation. The degree of inhibition over control at all the four concentrations of kinetin remained significant at p=0.001 level (**Table 18, Figs. 19a & b**).

IAA

IAA also inhibited both the phenomena in comparison (**Table 18**). However, unlike pollen germination the tube

elongation was not influenced by 1 ppm of IAA **(Table 18).** Inhibition of pollen germination and tube elongation increased with the increasing concentration of IAA **(Table 18, Figs. 19a & b).**

GA_3

All the four concentrations (1, 5, 10 and 25 ppm) of GA_3 had significant (p=0.001) inhibitory effect on pollen germination **(Table 18, Figs. 19a & b).** The pollen tube elongation was, however, not affected by these concentrations (1, 5, 10 and 25 ppm) of GA_3 **(Table 18, Figs. 19a & b).**

Sensitivity of Pollen to Radiation and Modulation of Radiation Response with Growth Hormones

S. khasianum

Effect of Gamma Radiation

Table 19a presents data on the effect of various doses (1-800 krad) of gamma radiation on pollen germination and pollen tube elongation in S. khasianum. Pollen germination in pollen irradiated with 1-100 krad doses of gamma rays was not significantly different from control. Gamma rays at 200 krad dose, however, stimulated pollen germination by 18.62% over control **(Table 19a, Fig. 20.1).** Gamma rays doses 500-800 krad inhibited pollen germination and the inhibition increased with the

increasing radiation dose **(Table 19a, Fig. 20.1).** Total inhibition of pollen germination occurred at 800 krad. The LD_{50} for S. khasianum pollen germination was between 600-700 krad **(Table 19a, Fig. 20.1).**

Except 1 krad dose of gamma radiation, which did not influence tube elongation, all other doses (50-800 krad) of gamma rays inhibited pollen tube elongation in S. khasianum **(Table 19a, Fig. 20.1).** The degree of inhibition increased with the increasing dosage. Unlike pollen germination, LD_{50} for pollen tube elongation was between 200-500 krad of gamma radiation **(Table 19a, Fig. 20.1).**

Table 19b gives data on the effect of various doses (1-800 krad) of gamma radiation on the occurrence of pollen tubes of varying lengths (15-375 µm). In control and 1 krad irradiated pollen smallest pollen tubes measured 151-225 µm while the longest tubes ranged between 301-375 µm. On the other hand, maximum pollen tubes in 600 and 700 krad irradiated pollen measured 15-75 µm. No pollen tubes at these doses measured more than 150 µm **(Table 19b).** Thus while the number of short pollen tubes increased with the increasing gamma rays doses, the frequency of long pollen tubes decreased with the increasing radiation dose **(Table 19b).**

Effect of Growth Hormones

Table 20 shows the effects of growth hormones (kinetin, IAA and GA_3) on pollen germination and pollen tube elongation in S. khasianum. In control 65.89% of pollen germinated and pollen tubes attained a size of 254.40 µm at the end of two hours of incubation **(Table 20, Figs. 21.1a & b).** These control pollen tubes revealed relatively higher amount of insoluble polysaccharides in the apical region while sub-apical and distal region showed moderate amount of insoluble polysaccharides **(Table 21, Fig. 22.1a).** Protein content was high throughout the control pollen tubes **(Table 21, Fig. 22.2a)** while nucleic acids (RNA) content was moderate **(Table 21, Fig. 22.3a).**

Low concentration (1 ppm) of kinetin had no effect on pollen germination and pollen tube elongation of S. khasianum although higher concentrations (5, 10 and 25 ppm) significantly decreased pollen germination and pollen tube elongation **(Table 20, Figs. 21.1a & b).** Inhibition of germination and tube growth over control increased with the increasing concentrations of kinetin to the extent that no pollen germinated at 25 ppm of kinetin **(Table 20, Figs. 21.1a & b).** Kinetin (10 ppm) treated pollen tubes were richer in their insoluble polysaccharides and protein content in comparison to control tubes **(Table 21, Figs. 22.1b & 22.2b).** No marked difference was evident in RNA content of control and

kinetin (10 ppm) treated pollen tubes **(Table 21, Fig. 22.3b).**

Similar to kinetin, 1 ppm of IAA had not effect on pollen germination and pollen tube elongation of S. khasianum **(Table 20).** Higher concentrations (5, 10 and 25 ppm) of IAA, however, inhibited both the processes **(Table 20, Figs. 21.1a& b).** The degree of inhibition increased with the increasing concentrations of IAA **(Table 20).** IAA (10 ppm) treated pollen tubes had more polysaccharides than control pollen tubes **(Table 21, Fig. 22.1c),** but were deficient in protein **(Table 21, Fig. 22.2c).** The apical region of IAA treated pollen tubes although had lesser nucleic acids (RNA) than control, the RNA content of sub-apical and distal region resembled control **(Table 21, Fig. 22.3c).**

Amongst the three growth hormones (GA_3, IAA and kinetin) S. khasianum pollen was least sensitive to GA_3. GA_3 at 1,5 and 10 ppm had no effect on pollen germination and pollen tube growth **(Table 20, Figs. 21.1a & b).** But 25 ppm of GA_3 inhibited both the processes in comparison to control **(Table 20, Figs. 21.1a & b).** The insoluble polysaccharide content in the apical and distal region of GA_3 (10 ppm) treated pollen tubes resembled control pollen tubes **(Table 21, Fig. 22.1d)** but sub-apical region revealed more insoluble polysaccharides than control

tubes **(Table 21, Fig. 22.1d).** In GA_3 treated pollen tubes, protein was less in comparison to control tubes **(Table 21, Fig. 22.2d)** but nucleic acid (RNA) content in the pollen tubes of GA_3 treated pollen resembled control **(Table 21, Fig. 22.3d).**

Modulation of Radiation Response

Table 20 gives data on the modulation response of growth hormones (kinetin, IAA and GA_3) on germination and tube growth of 600 krad (LD_{50} dose) irradiated pollen of S. khasianum. Unirradiated control pollen exhibited 65.85% germination while only 37.50% of 600 krad irradiated pollen germinated **(Table 20, Fig. 21.1a).** Pollen tubes of unirradiated pollen attained a length of 254.40 µm while those of irradiated pollen were 79.20 µm long **(Table 20, Fig. 21.1b).** Unlike control pollen tubes (unirradiated), the irradiated pollen tubes revealed very high intensity of insoluble polysaccharide and protein staining throughout the tubes **(Table 21, Fig. 22.1e, 22.2e).** Nucleic acids (RNA) staining intensity in irradiated pollen tubes was less than control (unirradiated) pollen tubes in the apical and distal regions but resembled control tubes in sub-apical region **(Table 21, Fig. 22.3e).**

Kinetin at 1 and 5 ppm although did not affect germination of 600 krad irradiated pollen of S. khasianum,

it further enhanced radiation injury to pollen tube elongation **(Table 20, Figs. 21.a & b).** 10 ppm of kinetin further reduced both the processes in irradiated pollen **(Table 20, Figs. 21.1a & b).** No germination of irradiated pollen occurred at 25 ppm of kinetin **(Table 20, Fig. 21a).** Insoluble polysaccharides and protein contents in 600 krad + 10 ppm of kinetin treated pollen tubes did not differ from irradiated control pollen tubes **(Table 21, Fig. 22.1f & 22.2f).** Nucleic acids (RNA) staining in the apical and distal regions of 600 krad + 10 ppm of kinetin treated pollen tubes was more intense compared to 600 krad irradiated pollen tubes although the intensity in sub-apical region resembled 600 krad irradiated pollen tubes **(Table 21, Fig. 22.3f).**

Germination of 600 krad irradiated pollen was not affected by 1 and 5 ppm of IAA **(Table 20, Fig.21.1a).** However, 10 ppm and 25 ppm of IAA further sensitized the inhibitory effects of gamma radiation on pollen germination of S. khasianum **(Table 20, Fig. 21.1a).** Pollen tube elongation, on the other hand, was adversely affected by all the concentrations (1, 5, 10 and 25 ppm) of IAA **(Table 20, Fig. 21.1b).** 600 krad + 10 ppm of IAA treated pollen tubes and 600 krad irradiated tubes, however, did not differ in their insoluble polysaccharides, protein and RNA contents **(Table 21, Figs. 22.1g, 22.2g & 22.3g).**

S. khasianum pollen irradiated with 600 krad of gamma rays exhibited partial recovery of germination when treated with 5 and 10 ppm of GA_3 **(Table 20, Fig.21.1a)** GA_3 at 1 and 25 ppm had no significant effect on germination of 600 krad irradiated pollen **(Table 20, Fig.21.1a).** Pollen tube elongation of irradiated (600 krad) pollen was, however, not affected by GA_3 (1, 5, 10 and 25 ppm). 600 krad + 10 ppm of GA_3 treated pollen tubes resembled 600 krad irradiated pollen tubes in their insoluble polysaccharides and protein content **(Table 21, Figs. 22.1h & 22.2h).** The nucleic acid (RNA) content in sub-apical and distal region of 600 krad + 10 ppm GA_3 treated tubes though resembled 600 krad irradiated tubes, a slight increase of RNA content was evident in the apical region of 600 krad + 10 ppm GA_3 treated tubes over irradiated pollen tubes **(Table 21, Fig. 22.3h).**

S. marginatum

Effect of Gamma Radiation

Table 22a gives data on the effect of gamma radiation (1-800 krad) on pollen germination and pollen tube growth of S. marginatum. In control, 64.31% of pollen germinated **(Table 22a, Fig. 20.2).** Low doses (1, 50 and 100 krad) of gamma radiation had no significant effect on pollen germination **(Table 22a, Fig.20.2).** 200 krad of gamma rays stimulated pollen germination over control

(Table 22a, Fig.20.2). Higher doses of radiation (500-800 krad), however, inhibited pollen germination and the degree of inhibition increased with the increasing dose **(Table 22a, Fig. 20.2).** No pollen germinated in 800 krad of gamma radiation **(Table 22a, Fig.20.2).** LD_{50} for pollen germination in S. marginatum ranged between 500 and 600 krad **(Table 22a, Fig.20.2).**

The elongation of pollen tube was though less than control in tubes given low doses of gamma radiation (1-100 krad) the inhibition was not significant statistically **(Table 22a, Fig. 20.2).** Higher doses of gamma radiation (200-800 krad), however, significantly inhibited pollen tube growth and the degree of inhibition increased with the increasing dose **(Table 22a, Fig.20.2).** LD_{50} for pollen tube elongation ranged between 200 and 500 krad **(Table 22a, Fig. 20.2).**

Data on the effect of gamma radiation (1-800 krad) on the incidence of pollen tubes of varying sizes is given in **Table 22b.** Control and 1 krad treated pollen produced longest pollen tubes measuring 376-450 µm **(Table 22b).** Increasing doses of gamma rays inhibited development of long pollen tubes and increased incidence of short pollen tubes **(Table 22b).**

Effect of Growth Hormones

Table 23 gives data on the effect of growth hormones (kinetin, IAA and GA_3) on pollen germination and pollen tube growth of S. marginatum. In control 81.46% of pollen germinated and pollen tubes measured 305.70 µm **(Table 23, Figs. 21.2a & b).** Cytochemical investigation of control pollen tubes revealed faint staining of insoluble polysaccharide throughout the pollen tubes **(Table 24, Fig. 23.1a).** These pollen tubes also showed moderate staining of protein and nucleic acid (RNA) in apical, sub-apical and distal regions of pollen tubes **(Table 24, Figs.** 23.2a & **23.3a).**

All the concentrations (1, 5, 10 and 25 ppm) of kinetin significantly inhibited pollen germination and pollen tube elongation in S. marginatum **(Table** 23, **Figs. 21.2a & b).** Compared to control pollen tubes, kinetin (10 ppm) treated pollen tubes exhibited higher intensity of insoluble polysaccharides staining **(Table 24, Fig.** 23.1b). Similarly, protein staining in kinetin treated pollen tubes was more in comparison to control pollen tubes **(Table 24, Fig. 23.2b).** Nucleic acids (RNA) staining in kinetin treated pollen tubes did not differ from control pollen tubes **(Table 24, Fig. 23.3b).**

Pollen germination and pollen tube elongation of S. marginatum were inhibited by IAA **(Table** 23, Figs.21.2a & **b).** But unlike pollen germination pollen tube growth was

not affected by 1 ppm of IAA **(Table 23, Fig.21.2b).** Pollen tubes treated with 10 ppm of IAA revealed higher intensity of insoluble polysaccharides staining compared to control pollen tubes **(Table 24, Fig. 23.1c).** The protein staining of IAA treated pollen tubes resembled control pollen tubes **(Table 24, Fig. 23.2c).** Further, though nucleic acid (RNA) staining of IAA treated pollen tubes, in apical and distal region resembled control tubes, it was higher than control in sub-apical region **(Table 24, Fig. 23.3c).**

All the four concentrations (1, 5, 10 and 25 ppm) of GA_3 inhibited pollen germination of S. marginatum but pollen tube elongation was not affected **(Table 23, Figs. 21.2a & b).** Control pollen tubes and 10 ppm GA_3 treated pollen tubes did not differ in their staining for insoluble polysaccharides and protein **(Table 24, Figs. 23.1d & 23.2d).** Compared to control pollen tubes the GA_3 treated tubes revealed more nucleic acid (RNA) in the sub-apical and distal region but apical region of GA_3 treated pollen tubes resembled control pollen tubes in their RNA content **(Table 24, Fig. 23.3d).**

Modulation of Radiation Response

Data on germination and tube elongation of 500 krad irradiated and growth hormones (kinetin, IAA and GA_3) treated pollen of S. marginatum are given in **Table 23.** Unirradiated pollen while exhibited 81.46% germination,

only 37.49% 500 krad (LD_{50}) irradiated pollen germinated **(Table 23, Fig.21.2a).** Unirradiated pollen tubes measured 305.70 µm but 500 krad irradiated pollen developed only 150.90 µm long pollen tubes **(Table 23, Fig.21.2b).** Compared to unirradiated control, the insoluble polysaccharides staining which was more in sub-apical and distal regions of irradiated pollen tubes resembled control in the apical region **(Table 24, Fig. 23.1c).** The intensity of protein staining in the tubes of irradiated pollen was much higher than control pollen tubes **(Table 24, Fig. 23.2e)** but nucleic acid (RNA) staining of irradiated pollen tubes resembled control (unirradiated) pollen tubes **(Table 24, Fig. 23.3e).**

Low concentrations (1 and 5 ppm) of kinetin had no effect on germination of 500 krad irradiated S. marginatum pollen **(Table 23, Fig.21.2a).** But higher concentrations (10 and 25 ppm) of kinetin further accentuated radiation-induced inhibition of pollen germination. Kinetin at 1 ppm though did not affect pollen tube elongation in 500 krad irradiated pollen, higher concentrations (5, 10 and 25 ppm) of kinetin further reduced elongation of irradiated pollen tubes **(Table 23, Fig.21.2b).** 500 krad + 10 ppm kinetin treated pollen tubes revealed more insoluble polysaccharides than 500 krad irradiated pollen tubes **(Table 24, Fig. 23.1f).** Protein staining of 500 krad + 10 ppm of kinetin treated pollen tubes, however,

resembled 500 krad irradiated pollen tubes **(Table 24, Fig. 23.2f).** Pollen tubes given 500 krad + 10 ppm kinetin revealed lesser nucleic acids (RNA) than 500 krad irradiated tubes **(Table 24, Fig. 23.3f).**

Low concentrations (1 and 5 ppm) of IAA had no significant effect on germination of 500 krad irradiated pollen of S. marginatum but higher concentrations (10 and 25 ppm) of IAA further increased radiation-induced inhibition of pollen germination **(Table 23, Fig.21.2a).** One ppm of IAA though did not alter pollen tube elongation in irradiated (500 krad) pollen higher concentrations (5, 10 and 25 ppm) of IAA further sensitized radiation-induced injury to tube elongation **(Table 23, Fig. 21.2b).** Compared to pollen tubes of irradiated (500 krad) pollen, 500 krad + 10 ppm of IAA treated pollen tubes revealed more insoluble polysaccharides but lesser protein and nucleic acid (RNA)**(Table 24, Figs. 23.1g, 23.2g & 23.3g).**

All the concentrations (1, 5, 10 and 25 ppm) of GA_3 did not alter significantly germination and tube elongation in 500 krad irradiated S. marginatum pollen **(Table 23, Figs. 21.2a & b).** Tubes given 500 krad + 10 ppm of GA_3 although revealed more insoluble polysaccharides and lesser protein than 500 krad irradiated pollen tubes **(Table 24, Figs. 23.1h & 23.2 h),** resembled irradiated pollen tubes **(Table 24, Fig. 23.3h)** in their RNA content.

DISCUSSION

In the present study, pollen of S. khasianum and S. marginatum could germinate in a wide range of pH (5.0-7.5). Maximum pollen germination and pollen tube elongation in both the species, however, occurred at pH 6.5. Higher pH decreased both the processes. Higher number of long pollen tubes also occurred at pH 6.5 in both the species. Pollen tube growth in both the species was, however, quite sensitive to changes in pH of the medium as was evident by the occurrence of different groups of pollen tubes at various pH. According to Brewbaker and Kwack (1964) pollen of many plant species usually exhibit best growth between pH 5.0-7.0. Shivanna and Johri (1985) stated that although pollen grains of each taxon apparently have an optimal pH range, slight deviations seldom have a drastic effect. However, according to the present study, slight shift in pH below or above pH 6.5 greatly reduced pollen germination and pollen tube growth. Such exacting requirement of pH (6.5) for optimum pollen germination and pollen tube growth is also reported in Vinca rosea (Bandyopadhya and Mukerjee, 1977). The ability of pollen to germinate in a wide range of pH had been attributed to the buffering action of pollen diffusates which shifts pH to its optimum requirement (Shivanna and Johri, 1985).

Temperature plays an important role in pollen germination and pollen tube elongation (Johri and Vasil, 1961). S. marginatum pollen although germinated within temperature range of 20-35°C, optimum pollen germination and pollen tube growth occurred at 30°C. At higher temperature (35°C) pollen tube elongation was drastically reduced and large amount of pollen bursting was evident. Maximum frequency of long pollen tubes was evident at 30°C to be closely followed by at 25°C. Such bursting of pollen due to high temperature had also been reported by early workers in some plant species (Smith, 1942; Vasil and Bose, 1959). Similar to S. marginatum, S. khasianum and S. indicum also require 30°C for optimal pollen germination and pollen tube growth (Ravindran and Chauhan, 1980). Pinus kesiya, a gymnosperm, also requires 30°C for optimal pollen tube elongation (Katiyar, 1991).

Sucrose concentrations are known to influence pollen germination and pollen tube elongation. The present investigation revealed that in S. marginatum pollen germination and pollen tube growth was retarded in absence of sucrose. Addition of sucrose (10-20%) greatly improved pollen germination and pollen tube elongation. Equally, good pollen germination, pollen tube elongation and more development of long pollen tubes occurred at 10-20% of sucrose, in the present study. Absence of sucrose not only resulted in poor pollen germination and pollen tube

elongation but also caused bursting of pollen in large numbers and increased the frequency of short pollen tubes. The optimum concentration of sucrose varies from species to species and 2-celled pollen require 10-20% sucrose (Shivanna and Johri, 1985). Pollen of Solanum aviculare, S. laciniatum, S. indicum and S. khasianum also require 15-20% of sucrose (Ravindran and Chauhan, 1980). But S. sisymbrifolium pollen require only 10% sucrose (Kuruvilla et al., in press) for optimum germination and tube elongation. Thus Solanum species have specific requirement of sucrose concentration for optimum growth of pollen tube. However, in the present study 10-20% of sucorse was equally effective for germination and elongation of pollen tubes of S. marginatum. Thus it seems that S. marginatum pollen are not very specific in their sucrose requirement. Sugars in the culture medium provide nutrition to the germinating pollen and also regulate osmotic pressure of the medium (Johri and Vasil, 1961). Profuse bursting in absence or at very low concentrations of sugar has been reported in literature (Brink, 1924; De Bruyn, 1966). The present study also supports these findings. Bursting of pollen is believed to be due to excessive uptake of water (De Bruyn, 1966) and it can be prevented by altering the osmotic concentration of the medium (Vasil, 1960). The effect of sucrose in regulation of pollen bursting could be because sucrose functions as osmoticum.

Incorporation of boric acid (100-400 ppm) in the germinating medium though improved germination of S. marginatum pollen over control, the mean value between different concentrations of boric acid did not differ significantly. This suggests that significant germination of S. marginatum pollen can be achieved in a wide range (100-400 ppm) of boric acid in the germinating medium. On the other hand, the requirement of boric acid concentration for pollen tube elongation in S. marginatum is limiting and maximum tube elongation occurred at 100-200 ppm of boric acid. Thereafter it declined. Similarly, the frequency of long pollen tubes increased with the addition of 100 and 200 ppm of boric acid in the germinating medium. However, the frequency of long pollen tubes decreased at 300 and 400 ppm of boric acid and short pollen tubes frequency increased. These results suggest that S. marginatum pollen require 100-200 ppm of boric acid for optimum germination and tube elongation. Other species of Solanum (S. aviculare, S. laciniatum, S. indicum and S. khasianum) also require 100-200 ppm of boric acid for maximum pollen germination and pollen tube elongation (Ravindran and Chauhan, 1980). But S. sisymbrifolium pollen require more exacting amount of boric acid for optimum germination and tube growth. Higher concentration (300 ppm) of boric acid proved inhibitory for germination of pollen of these species of Solanum

(Ravindran and Chauhan, 1980; Kuruvilla et al., in press) but it was not so in case of S. marginatum pollen in the present study. The effect of boron on pollen germination and pollen tube elongation is manifold : (i) it forms sugar-borate complexes which increase absorption, translocation and metabolism of sugars (Vasil, 1964), (ii) it increases oxygen uptake (O'Kelley, 1957), (iii) it is involved in the synthesis of pectin materials for the wall of actively growing pollen tubes (Stanley and Loewus, 1964) and (iv) it enhances the chemotropic response of pollen to calcium (Mascarenhas and Machlis, 1964). Thus boron action is complex.

Pollen of S. khasianum and S. marginatum have differential response to various concentrations of calcium. Pollen germination in S. khasianum was insensitive to 100 ppm of calcium eventhough, pollen tube elongation was significantly enhanced. Higher concentrations (200-400 ppm) of calcium greatly reduced pollen germination in S. khasianum but pollen tube growth was not affected vis-a-vis control. On the other hand, pollen germination in S. marginatum was significantly reduced when 100 ppm of calcium was added to the germinating medium although pollen tube growth remained insensitive to this concentration. Higher concentrations (200-400 ppm) of calcium further decreased both pollen germination and pollen tube elongation in S. marginatum.

Thus in S. marginatum better pollen germination and tube elongation occurred in medium devoid of calcium but S. khasianum pollen required addition of 10 ppm of calcium for optimum germination and tube growth. Thus, whereas pollen of S. marginatum do not require calcium for germination and tube growth, S. khasianum pollen require exogenous supply of calcium in small quantity for optimum germination and tube growth. The requirement of exogenous supply of calcium for maximum pollen tube growth is also supported by the occurrence of more long pollen tubes in medium supplemented with low concentrations of calcium. The higher concentrations of calcium caused higher frequency of short pollen tubes in S. khasianum. In S. marginatum the frequency of long pollen tubes was higher in absence of calcium and was much reduced in medium incorporated with calcium. Thus the calcium requirement of the two species are different. Calcium plays important roles in pollen germination and pollen tube growth. It gives rigidity to the pollen tube wall (Kwack, 1967), controls permeability of the tube membrane (Dickinson, 1967) and maintains the growth of pollen tube tip by controlling unidirectional movement of vesicles towards the tip and fusion of vesicles with the plasma membrane (Weisenseel and Jaffe, 1976; Reiss and Herth, 1979; Picton and Steer, 1983; Polito, 1983a,b). Some workers have also suggested the involvement of calcium in protein phosphorylation and enzyme activity in pollen tubes

(Nakamura, 1978; Polya et al., 1986). Incorporation of calcium into the germinating medium was thought to be necessary for optimum pollen germination and pollen tube growth in vitro (Brewbaker and Kwack, 1963) hence 300 ppm of calcium has been routinely used for the purpose. Pollen grains of many taxa, however, do not require exogenous supply of calcium for germination and tube growth (Glenk et al., 1969). This is believed to be due to the presence of higher levels of endogenous calcium in the pollen or due to contamination of the germinating medium (Shivanna and Johri, 1985).

In the present investigation pollen of S. khasianum and S. marginatum responded differently to various concentrations of magnesium. Incorporation of magnesium in the germinating medium for S. khasianum pollen inhibited both germination and tube elongation and the degree of inhibition increased with the increasing concentration. Occurrence of short pollen tubes also increased with the increasing concentration while medium devoid of magnesium supported more longer tubes in S. khasianum. On the other hand, in S. marginatum incorporation of magnesium (100-400 ppm) in the medium improved both pollen germination and pollen tube elongation. The degree of stimulation increased upto 200 ppm, the concentration higher than this reduced degree of

stimulation in both the processes. Similarly, magnesium at 200 ppm increased the occurrence of long pollen tubes. Magnesium in the germinating medium acts as an accelerating factor for the stimulatory effect of calcium (Brewbaker and Kwack, 1963). Whereas pollen of many plant species require magnesium for pollen germination and tube elongation (Shivanna, 1977; Johri and Shivanna, 1977; Shivanna and Heslop-Harrison, 1981) there are many other plant species which do not require exogenous magnesium for germination and tube growth (Cook and Walden, 1965; Pfahler, 1968; Pfahler and Linskens, 1973; Hoekstra, 1973; Shivanna et al., 1978; Shivanna and Heslop-Harrison, 1981). S. khasianum while belongs to the first category of plants, S. marginatum belongs to the second category of plants.

Pollen of S. khasianum and S. marginatum respond differently to various concentrations of potassium. Pollen germination in S. khasianum was inhibited by potassium but stimulated in S. marginatum. Potassium although inhibited pollen tube elongation in S. khasianum, the pollen tube growth was unaffected in S. marginatum. Pollen germination and pollen tube elongation decreased with the increasing concentration of potassium in S. khasianum. Similarly while frequency of long pollen tubes reduced, the frequency of short pollen tubes increased with

the increasing concentration of potassium in S. khasianum. In S. marginatum maximum pollen germination occurred at 200 ppm of potassium and, thereafter, declined. Although, potassium had no significant effect on pollen tube elongation in S. marginatum the frequency of long pollen tubes was higher at 200 ppm of potassium. Like magnesium, potassium acts as a supporting ion for the calcium effect (Brewbaker and Kwack, 1963). There are reports to suggest that pollen of some plant species require potassium for germination and tube growth (Shivanna, 1977; Shivanna and Johri, 1977; Shivanna and Heslop-Harrison, 1981), while pollen of many other plant species do not require potassium (Pfahler, 1965; Glenk et al., 1969; Rao and Ong, 1972; Ferrari and Wallace, 1975; Roberts et al., 1983). According to Brewbaker and Kwack (1963) pollen tubes do not require magnesium or potassium for growth, but these cations maximise the association of calcium in the cell wall. The present findings indicated that pollen tube elongation in S. khasianum does not require magnesium and potassium. On the contrary, magnesium and potassium inhibited pollen tube elongation. However, in S. marginatum pollen tube growth improved on addition of magnesium while potassium had no influnce. Therefore, pollen of S. khasianum and S. marginatum showed differential response to magnesium and potassium.

Gelling of the germinating medium with bacto-agar was beneficial for pollen germination and tube elongation in both S. khasianum and S. marginatum. Significant enhancement of pollen germination and pollen tube elongation over control occurred when 1% agar was incorporated in the pollen germination medium for both the species. However, the response of both the species to various concentration of agar differed and the effect was more pronounced in S. khasianum. Higher concentrations (1.4-18%) of agar inhibited both the processes in both the species investigated. Controlled imbibition or hydration of pollen is important for the subsequent processes of germination and tube elongation (Shivanna and Johri, 1985; Hoekstra and Bruinsma, 1975; Bar-Shalom and Mattsson, 1977; Shivanna and Heslop-Harrison, 1981; Shivanna et al., 1983). Gelling of the medium controls hydration (Shivanna and Johri, 1985) which could be the reason for gelling-induced stimulation of pollen germination and tube elongation in both the species.

In the present study, the optimum factors (both physical and nutritional) for pollen germination and pollen tube elongation in S. khasianum and S. marginatum determined in the preceeding experiments were further substantiated by germinating pollen in different media. In S. khasianum and S. marginatum pollen germination in

Brewbaker and Kwack's (1963) medium was less than that observed in control medium (Sucrose + agar). However, pollen tube elongation in Brewbaker and Kwack's (1963) medium was better than in control. Modified Brewbaker and Kwack's medium (present study) supported maximum pollen germination and pollen tube growth. This was also supported by the occurrence of higher frequency of long pollen tubes when pollen was germinated in the modified medium. In the present study control medium which contained simple sugar (15%) and agar (1%) supported good pollen germination and tube elongation in both the species. Thus these results also support the findings of Swaminathan (1955) that sugar-agar/gelatin is suitable for germination and tube growth of most Solanum species.

In the present investigation germination of S. khasianum and S. marginatum pollen was not influenced by 1-100 krad doses of gamma rays. In both the species, however, 200 krad of gamma rays stimulated pollen germination. Thereafter pollen germination percentage declined and the degree of inhibition increased with the increasing dosage of gamma rays. LD_{50} for S. khasianum and S. marginatum pollen germination ranged between 600-700 krad and 500-600 krad, respectively. However, LD_{100} for both the species proved to be 800 krad. Pollen tube elongation in both the species was differently in

by various doses of gamma rays, the degree of inhibition increased with the increasing dosage of radiation. Although pollen germination in both the species was stimulated by 200 krad, this dose inhibited pollen tube elongation. Inhibition of pollen tube growth by gamma radiation, in both the species, is also supported by the occurrence of increased incidence of short pollen tubes at higher doses of radiation. Long pollen tubes were more in pollen irradiated with lower doses of gamma rays and control. In both the species LD_{50} for pollen tube growth ranged between 200-300 krad. Pollen grain size has a correlation with radiosensitivity; larger grains being more radiosensitive (Brewbaker and Emery, 1962). In plant kingdom pollen diameter varies between 10-200 µm. In the present study pollen grains of S. khasianum and S. marginatum measure approximately 25 µm and 30 µm respectively and they appear to be quite resistant to gamma radiation. Present findings support the statement of Brewbaker and Emery (1962) that germination and tube growth are generally inhibited only following massive radiation doses; LD_{50} ranging upto 550 krad. High resistance of pollen to massive dose of gamma radiation has been attributed to the radioresistance of glutamate dehydrogenase, glucose-6-phosphate dehydrogenase, α - esterase and acid phosphatase in high dose radiation-inhibited pollen tube growth of Lilium regale and Beta

vulgaris (Georgieva and Atanassov, 1986). Similar factor may be responsible for high resistance of S. khasianum and S. marginatum pollen to gamma radiation. Some earlier workers have shown that in Solanum torvum (Chauhan and Katiyar, 1990), pines (Zelles and Seibold, 1976; Katiyar and Chauhan, 1978a,b; Katiyar, 1991) and Pseudotsuga menziesii (Livingston and Stettler, 1973) low dose of ionizing radiation stimulated pollen germination and pollen tube growth. However, in the present study pollen tube elongation was not stimulated by any dose, though pollen germination was significantly stimulated over control. This may be due to difference in radiosensitivity of different plant species (Brewbaker and Emery, 1962). Radiation-induced inhibition of pollen tube growth has been attributed to the damage of cell membrane (Brewbaker and Emery, 1962; Pfahler, 1971, Visser and Oost, 1981). Thus in the present investigation radiation doses 50-800 krad may have damaged cell membrane.

Kinetin and IAA inhibited pollen germination and pollen tube growth of S. khasianum and S. marginatum. The degree of inhibition increased with the increasing concentrations of kinetin and IAA. Pollen of S. khasianum and S. marginatum, however, differed in their response to GA_3. Both pollen germination and pollen tube growth of S. khasianum were insensitive to low concentrations (1-10

ppm) of GA_3 while higher concentration (25 ppm) inhibited both the processes. On the otherhand, pollen germination of S. marginatum was inhibited by all the concentrations (1-25 ppm) of GA_3 but pollen tube elongation was not affected. These findings further confirm the findings of Ravindran and Chauhan (1986) that kinetin, IAA and GA_3 inhibit both pollen germination and pollen tube elongation in S. khasianum. These growth hormones also inhibit pollen germination and pollen tube elongation in many other plant species (De Bruyn, 1966 ; Tsukamoto and Matsubara, 1968; Taylor, 1972; Sondheimer and Linskens, 1974; Mehan and Malik, 1975; Nakamura, 1978; Ravindran and Chauhan, 1986).

The inhibitory effect of growth hormones (kinetin and IAA) on pollen germination and pollen tube growth in the present study was also reflected in the cytochemistry of treated pollen. Many workers have demonstrated that polysaccharides, protein and nucleic acids are synthesized and utilized by germinating pollen for its tube growth (Stanley and Linskens, 1974; Malik and Gupta, 1976; Sharma, 1978; Chhabra and Malik, 1978; Capkova et al., 1987, 1988). Present investigation revealed that growth hormones-induced inhibition of pollen tube elongation in both the Solanum species was accompanied by increased intensity of insoluble polysaccharides in the pollen tubes

implying thereby that effective utilization of polysaccharides in kinetin and IAA treated pollen tubes was less than control. The inhibitory action of auxins on root growth is attributed to the persistent loosening of the intermicellar bonds in the cellulose wall which prevents the formation of intermicellar linkages by new cellulose microfibrils (Burstrom, 1957). Oxygen uptake by stem segments of pea (Katsumi, 1963) and cell suspension cultures of tobacco (Bergman, 1964) was considerably reduced by kinetin. This is due to the inhibition of glycolytic pathway of carbohydrate metabolism (Bergman, 1964). These may be the reasons for kinetin, IAA-induced pollen tube growth inhibition and accumulation of PAS positive compounds in pollen tubes in the present study as well. Moreover in kinetin-inhibited stem segment elongation of Avena, kinetin inhibits the full development of invertase (involved in cleavage of sucrose to glucose and fructose) and enhances the decay of this enzyme (Jones and Kaufman, 1971). This may be the reason why kinetin treated pollen failed to utilise sucrose in the germinating medium for tube elongation. GA_3 treatment had no influence on the intensity of insoluble polysaccharides in the metabolically active region (apical) of pollen tubes suggesting that in GA_3 treated tubes of both the species utilization of polysaccharides was not affected. This may be the reason why pollen tube elongation in both the

species was not influenced by GA_3.

Kinetin inhibited pollen tubes of S. khasianum and S. marginatum displayed marked increase in protein intensity. Protein synthesis is initiated during the early stage of pollen germination before the start of pollen tube elongation (Mascarenhas, 1975; Malik, 1985). Proteins formed during the activation phase of pollen germination are utilized for the synthesis of cell wall (Malik and Gupta, 1976; Capkova et al., 1987, 1988). The fact that protein intensity in kinetin inhibited pollen tubes of S. marginatum was more than control pollen tubes suggests that protein formed during activation phase could not be utilized by the pollen tubes. Hence pollen tubes remained shorter than control pollen tubes. Cytokinin caused inhibition of potato stolon elongation is suggested to be associated with poor utilization of proteins into enzymes (Skoog and Armstrong, 1970). High concentrations of kinetin also inhibit proteinase activity in the cotyledons of intact embryos of Squash (Penner and Ashton, 1967). Such a mechanism may also be responsible for kinetin-induced inhibition of pollen tube elongation since utilization of protein for the synthesis of certain enzymes involved in the biogenesis of cell wall polysaccharides in pollen tubes has been reported (Malik and Gupta, 1976). Protein intensity in the germinating

pollen tubes of S. marginatum was not influenced by IAA. However, IAA treated pollen tubes of S. khasianum showed reduced intensity of protein. According to Northen (1942) auxins bring about dissociation of the protein constituents of the cytoplasm which may bring about stimulation of respiration and growth. He also pointed out that if dissociation activities are carried out to the extreme, the stimulatory effect upon enzymes in respiration would be reversed by dissociation of essential constituents from enzymes and according to him this may be the cause of inhibition of growth by auxin and the concomitant inhibition of respiration. Such phenomenon of protein break ups due to IAA could be the reason for reduced intensity of protein and poor utilization of polysaccharides. GA_3 treated pollen tubes of S. khasianum exhibited reduced intensity of protein. However, GA_3 had no influence on protein content in pollen tubes of S. marginatum. In the present study GA_3 (10 ppm) had no influence on pollen germination and pollen tube growth of S. khasianum. Therefore, reduced intensity of protein in pollen tubes of S. khasianum as a result of GA_3 treatment is not clearly understood.

Nucleic acids, particularly, RNA are located at the tip of actively growing pollen tubes (Dashek and Rosen, 1962; Rosen, 1968; Rahaman and Patil, 1985).

In the present investigation, RNA intensity in the apical region of S. khasianum pollen tubes was not influenced by kinetin and GA_3. However, RNA intensity in the apical region of IAA treated pollen tubes was reduced in comparison to the apical region of control pollen tubes. However, sub-apical and distal regions resembled control pollen tubes in RNA staining. RNA in the apical region of S. marginatum pollen tubes was not influenced by kinetin, IAA and GA_3 treatment though some amount of variations were noted in other regions of the pollen tubes.

The observations made in the present investigation reveal that the cytochemical responses of pollen tubes of S. khasianum and S. marginatum to kinetin, IAA and GA_3 differ suggesting differential response of pollen of the two species to growth hormones. Wareing (1977) while discussing the effect of growth hormones on integration of activity in higher plants emphasized "the specificity of the response to a given growth substance is usually determined by the 'competence' or programming of the target tissue and which may be the reason for different effect of a growth hormone in different plants."

Pollen of S. khasianum and S. marginatum when irradiated with their respective LD_{50} dose of gamma rays for germination revealed higher amount of insoluble polysaccharides and protein in the pollen tubes than

unirradiated control. 600 krad irradiated pollen of S. khasianum revealed moderate amount of nucleic acids in the sub-apical region and very less in the apical and distal region. Irradiated pollen tubes of S. marginatum resembled unirradiated control in their nucleic acid (RNA) content. When irradiated pollen of S. khasianum (600 krad) and S. marginatum (500 krad) were germinated on medium supplemented with 10 ppm of kinetin and IAA, the radiation-induced inhibition of pollen tube growth was further aggravated. Compared to the tubes formed by irradiated pollen germinated on medium lacking in growth hormones, the tubes developed from pollen grains treated with radiation + 10 ppm kinetin and radiation + 10 ppm IAA elicited an increase in insoluble polysaccharides intensity. The increase in insoluble polysaccharides was prominent in pollen tubes of S. marginatum. In both the species, radiation + 10 ppm kinetin treated pollen revealed very high intensity of protein which was similar to control (irradiated) pollen tubes. Nucleic acid (RNA) staining in pollen tubes given radiation + 10 ppm kinetin, however, showed variable intensity in the two species. 600 krad + 10 ppm kinetin treated pollen tubes of S. khasianum revealed higher intensity of RNA in the apical and distal region while sub-apical region revealed identical intensity with irradiated control. On the otherhand, 500 krad + 10 ppm kinetin treated pollen tubes

of S. marginatum showed lesser intensity of nucleic acid (RNA) than control (irradiated) pollen tubes. Compared to irradiated control, 600 krad + 10 ppm IAA treated pollen tubes of S. khasianum revealed identical staining intensity of protein and nucleic acid (RNA) while 500 krad + 10 ppm IAA treated pollen tubes of S. marginatum showed lesser intensity of protein and nucleic acid (RNA). Thus pollen tube elongation in irradiated pollen of S. khasianum and S. marginatum appears to have been inhibited further by kinetin and IAA through lesser utilization/synthesis of polysaccharides, protein and nucleic acid (RNA). Although GA_3 did not influence pollen tube elongation in irradiated pollen of both the species cytochemical constituents in radiation + 10 ppm GA_3 treated pollen tubes were affected. The pollen tubes of 600 krad + 100 ppm GA_3 treated S. khasianum pollen resembled irradiated control pollen tubes in insoluble polysaccharides and protein content. Similarly, nucleic acid (RNA) in the sub-apical and distal region of 600 krad + 10 ppm GA_3 treated pollen tubes showed identical intensity with that of irradiated control pollen tubes. However, compared to irradiated control pollen tubes increased intensity of nucleic acid (RNA) was evident in the apical region of GA_3 treated pollen tubes of S. khasianum. 500 krad + 10 ppm GA_3 treated pollen tubes of

S. marginatum displayed higher intensity of insoluble polysaccharides, lesser intensity of protein and identical intensity of nucleic acid (RNA) in comparison with irradiated control pollen tubes. Thus sensitivity of cytochemical constituents to GA_3 in irradiated pollen tubes differs between the two species.

Table 2a. Effect of pH on pollen germination and pollen tube Growth in S. khasianum

pH	Pollen Germination (%)	Pollen Tube Length (μm)
5.0	40.13 ± 2.36	176.00 ± 0.86
5.5	41.81 ± 1.14	183.00 ± 0.82
6.0	40.19 ± 1.44	204.30 ± 0.87**
6.5	53.26 ± 3.56***	226.50 ± 1.34***
7.0	31.92 ± 3.88*	142.20 ± 0.66*
7.5	34.31 ± 1.33*	142.50 ± 0.59*
LSD (p=0.05)	7.38	31.20

± S.E.

* Significantly different from mean at pH 5.0 at p = 0.05

** Significantly different from mean at pH 5.0 at p = 0.01

*** Significantly different from mean at pH 5.0 at p = 0.001.

Table 2b. Effect of pH on pollen tube groups in S. khasianum

pH	Pollen tubes (%)						
	15-75 (μm)	76-150 (μm)	151-225 (μm)	226-300 (μm)	301-375 (μm)	376-450 (μm)	451-525 (μm)
5.0	20.00±1.10	28.00±0.75	24.00±1.62	18.00±1.33	10.00±1.55	0	0
5.5	10.00±0.63	28.00±0.45	22.00±1.33	30.00±1.20	10.00±0.63	0	0
6.0	8.00±0.74	26.00±1.02	32.00±1.60	22.00±1.17	12.00±1.47	0	0
6.5	16.00±0.40	28.00±1.33	10.00±0.89	20.00±0.63	8.00±0.75	10.00±1.10	8.00±0.98
7.0	24.00±1.50	38.00±1.60	22.00±1.47	12.00±0.75	4.00±0.80	0	0
7.5	20.00±0.89	34.00±0.80	40.00±0.63	6.00±0.49	0	0	0

± SE

Table 3a. Effect of calcium concentrations on pollen germination and pollen tube growth in S. khasianum

Calcium (ppm)	Pollen Germination (%)	Pollen Tube Length (μm)
0	66.31 ± 2.92	237.30 ± 0.53
1	68.32 ± 3.87	246.00 ± 1.05
10	73.18 ± 2.22**	304.50 ± 0.24***
25	65.13 ± 1.51	288.60 ± 0.37**
50	68.99 ± 2.37	266.70 ± 0.76*
100	63.80 ± 1.46	266.93 ± 0.14*
200	59.50 ± 3.33**	243.19 ± 0.26
300	57.05 ± 0.98***	237.30 ± 0.31
400	54.41 ± 2.59***	239.20 ± 0.27
LSD (p=0.05)	4.15	29.10

± S.E.

* Significantly different from control at p = 0.05

** Significantly different from control at p = 0.01

*** Significantly different from control at p = 0.001

Table 3b. Effect of calcium concentrations on pollen tube groups in S. khasianum

Calcium (ppm)	Pollen tubes (%)					
	15-75 (μm)	76-150 (μm)	151-225 (μm)	226-300 (μm)	301-375 (μm)	376-450 (μm)
0	0	0	14.00±0.58	70.00±0.88	13.00±0.74	3.00±0.23
1	0	0	22.00±0.84	66.00±0.78	10.00±0.53	2.00±0.16
10	0	0	4.00±0.33	60.00±0.41	34.00±0.58	2.00±0.21
25	0	0	15.00±0.23	65.00±0.50	18.00±0.50	2.00±0.31
50	0	4.00±0.33	31.00±1.26	55.00±1.08	10.00±0.47	0
100	15.00±1.62	30.00±1.60	13.00±1.67	33.00±1.50	11.00±1.20	0
200	19.00±1.55	31.00±2.24	23.00±1.85	21.00±1.10	6.00±1.17	0
300	21.00±0.40	39.00±1.47	21.00±1.83	19.00±2.79	0	
400	26.00±2.23	26.00±2.23	23.00±1.72	25.00±2.01	0	

± SE

Table 4a. Effect of magnesium concentrations on Pollen germination and pollen tube growth in S. khasianum

Magnesium (ppm)	Pollen Germination (%)	Pollen Tube Length (μm)
0	63.72 ± 4.56	184.50 ± 0.39
100	51.59 ± 3.39*	164.40 ± 0.63
200	43.13 ± 1.68**	156.30 ± 0.52*
300	39.97 ± 1.68***	128.40 ± 0.33**
400	32.35 ± 3.30***	111.90 ± 0.70***
LSD (p=0.05)	9.24	23.70

± S.E.

* Significantly different from control at p = 0.05

** Significantly different from control at p = 0.01

*** Significantly different from control at p = 0.001

Table 4b. Effect of magnesium concentrations on pollen tube groups in *S. khasianum*

Magnesium (ppm)	Pollen tubes (%)			
	15-75 (μm)	76-150 (μm)	151-225 (μm)	226-300 (μm)
0	3.00±0.15	23.00±0.52	60.00±0.49	14.00±0.73
100	2.00±0.20	59.00±0.71	30.00±0.82	9.00±0.31
200	7.00±0.26	58.00±0.63	27.00±0.50	8.00±0.25
300	28.00±0.66	57.00±0.63	13.00±0.30	2.00±0.20
400	31.00±0.77	55.00±0.54	11.00±0.41	3.00±0.15

± SE

Table 5a. Effect of potassium concentrations on pollen germination and pollen tube growth in *S. khasianum*

Potassium (ppm)	Pollen Germination (%)	Pollen Tube Length (μm)
0	81.23 ± 0.81	223.80 ± 0.75
100	75.08 ± 1.61*	196.20 ± 0.29*
200	62.86 ± 2.91***	189.60 ± 0.53*
300	53.96 ± 1.60***	179.10 ± 0.65**
400	52.02 ± 1.84***	167.40 ± 0.57***
LSD (p=0.05)	5.56	25.34

± S.E.

* Significantly different from control at p = 0.05

** Significantly different from control at p = 0.01

*** Significantly different from control at p = 0.001

Table 5b. Effect of potassium concentrations on pollen tube groups in S. khasianum

Potassium (ppm)	Pollen tubes (%)		
	15-150 (μm)	151-225 (μm)	226-300 (μm)
0	2.00 ± 0.40	58.00 ± 2.79	40.00 ± 2.97
100	38.00 ± 2.14	56.00 ± 1.50	6.00 ± 0.80
200	16.00 ± 0.49	60.00 ± 0.89	24.00 ± 1.02
300	28.00 ± 2.04	58.00 ± 1.60	14.00 ± 1.36
400	22.00 ± 1.16	64.00 ± 0.49	14.00 ± 0.80

± SE

Table 6a. Effect of gelling on pollen germination and pollen tube growth in S. khasianum

Agar (%)	Pollen Germination (%)	Pollen Tube Length (μm)
0	35.30 ± 4.02	93.90 ± 0.41
0.2	51.76 ± 2.61***	138.90 ± 0.55*
0.6	52.09 ± 2.30***	167.70 ± 0.56***
1.0	53.67 ± 1.51***	200.10 ± 0.65***
1.4	31.52 ± 1.19	152.40 ± 0.66**
1.8	27.17 ± 1.39*	147.00 ± 0.48*
LSD (p=0.05)	7.68	39.90

± S.E.

* Significantly different from control at p = 0.05

** Significantly different from control at p = 0.01

*** Significantly different from control at p = 0.001

Table 6b. Effect of gelling on pollen tube groups in S. khasianum

Agar (%)	Pollen tubes (%)				
	15-75 (μm)	76-150 (μm)	151-225 (μm)	226-300 (μm)	301-375 (μm)
0	52.00±2.04	40.00±1.41	6.00±0.80	2.00±0.40	0
0.2	10.00±0.63	58.00±2.04	30.00±2.28	2.00±0.40	0
0.6	4.00±0.49	46.00±1.62	36.00±1.35	14.00±1.02	0
1.0	0	32.00±0.98	34.00±2.06	32.00±1.16	2.00±0.40
1.4	16.00±1.49	42.00±1.94	28.00±1.16	12.00±1.16	2.00±0.40
1.8	12.00±1.60	46.00±1.02	40.00±2.06	2.00±0.40	0

± SE

Table 7a. Effect of pollen germination medium on Pollen germination and pollen tube growth in S. khasianum

Medium	Pollen Germination (%)	Pollen Tube Length (μm)
Control[1]	62.47 ± 1.42	144.60 ± 0.52
B & K[2]	51.66 ± 3.85**	198.90 ± 1.13*
MB & K[3]	82.17 ± 0.49***	266.10 ± 0.82***
LSD (p=0.05)	7.35	39.15

± S.E.

* Significantly different from control at p = 0.05

** Significantly different from the control at p = 0.01

*** Significantly different from control at p = 0.001

1 Control = Sucrose + Agar

2 B & K = Brewbaker and Kwack's medium (1963)

3 MB & K = Modified Brewbaker and Kwack's medium.

Table 7b. Effect of pollen germination medium on pollen tube groups in S. khasianum

Medium	Pollen tubes (%)				
	15-150 (μm)	151-225 (μm)	226-300 (μm)	301-375 (μm)	376-450 (μm)
Control[1]	39.00±1.30	42.00±1.13	16.00±0.85	3.00±0.30	0
B & K[2]	11.00±0.51	28.00±0.90	36.00±0.75	24.00±0.80	1.00±0.11
MB & K[3]	0	23.00±0.42	56.00±0.48	20.00±0.54	1.00±0.11

± SE

1 Control - Sucrose + Agar

2 B & K - Brewbaker and Kwack's medium (1963)

3 MB & K - Modified Brewbaker and Kwack's medium.

Table 8a. Effect of temperature on pollen germination and pollen tube growth in *S. marginatum*

Temperature (°C)	Pollen germination (%)	Pollen bursting (%)	Pollen tube length (μm)
20	43.55±1.65	0	132.90±0.26
25	54.81±1.77***	0	171.90±0.19***
30	61.67±1.73***	0	256.20±0.43***
35	49.30±1.75*	41.79±1.57	71.10±0.27***
LSD (p=0.05)	4.96		13.36

± SE

* Significantly different from mean at 20°C at p=0.05

*** Significantly different from mean at 20°C at p=0.001

Table 8b. Effect of temperature on pollen tube groups in S. marginatum

Temperature (°C)	Pollen tubes (%)					
	15-75 (μm)	76-150 (μm)	151-225 (μm)	226-300 (μm)	301-375 (μm)	376-450 (μm)
20	60.00±1.94	38.00±2.22	2.00±0.40	0	0	0
25	28.00±1.72	30.00±1.55	14.00±0.80	16.00±1.62	12.00±1.17	0
30	18.00±1.47	12.00±1.17	14.00±1.20	20.00±1.41	28.00±1.17	8.00±1.17
35	94.00±0.80	6.00±0.80	0	0	0	0

± SE

Table 9a. Effect of pH on pollen germination and pollen tube growth in S. marginatum

pH	Pollen Germination (%)	Pollen Tube Length (μm)
5.0	44.71 ± 2.96	78.60 ± 0.38
5.5	41.24 ± 1.94	110.38 ± 0.65*
6.0	53.27 ± 3.71*	112.50 ± 0.59**
6.5	69.16 ± 3.02***	178.50 ± 1.13***
7.0	57.88 ± 1.32**	151.80 ± 0.95 ***
7.5	50.22 ± 2.26	133.20 ± 0.85***
LSD (p=0.05)	7.73	27.75

± S.E.

* Significantly different from mean at pH 5.00 at p = 0.05

** Significantly different from mean at pH 5.00 at p = 0.01

** Significantly different from mean at pH 5.00 at p = 0.001.

Table 9b. Effect of pH on pollen tube groups in S. marginatum

pH	Pollen tubes (%)				
	15-75 (μm)	76-150 (μm)	151-225 (μm)	226-300 (μm)	301-375 (μm)
5.0	58.00±1.17	42.00±1.17	0	0	0
5.5	42.00±3.40	25.00±3.11	33.00±2.75	0	0
6.0	32.00±0.98	44.00±1.02	20.00±0.89	4.00±0.49	0
6.5	30.00±1.09	14.00±1.02	20.00±1.09	24.00±1.02	12.00±1.02
7.0	34.00±0.80	20.00±0.89	22.00±0.80	24.00±1.35	0
7.5	42.00±1.17	20.00±1.69	14.00±0.49	24.00±1.35	0

± SE

Table 10a. Effect of sucrose concentrations on pollen germination and pollen tube growth in S. marginatum

Sucrose (%)	Pollen germination (%)	Pollen bursting (%)	Pollen tube length (μm)
0	8.74±0.83	66.92±2.86	53.40±0.37
10	53.81±3.61***	0	110.70±0.58***
15	60.71±4.81***	0	125.40±0.65***
20	55.20±6.03***	0	122.40±0.70***
LSD (p=0.05)	12.83		28.05

± SE

*** Significantly different from control at p=0.001

Table 10b. Effect of sucrose concentrations on pollen tube groups in S. marginatum

Sucrose (%)	Pollen tubes (%)			
	15-75 (μm)	76-150 (μm)	151-225 (μm)	226-300 (μm)
0	90.00±1.02	10.00±1.09	0	0
10	36.00±1.20	44.00±1.36	20.00±1.90	0
15	46.00±0.49	24.00±2.15	20.00±1.67	10.00±0.89
20	46.00±0.80	20.00±1.41	26.00±0.80	8.00±1.17

± SE

Table 11a. Effect of boron concentrations on pollen germination and pollen tube growth in S. marginatum

Boron (ppm)	Pollen Germination (%)	Pollen Tube Length (μm)
0	50.88 ± 2.52	172.20 ± 0.33
100	66.60 ± 1.23***	194.00 ± 0.45*
200	72.50 ± 1.01***	195.10 ± 0.51*
300	68.34 ± 1.00***	181.20 ± 0.43
400	71.76 ± 1.43***	168.00 ± 0.23
LSD (p=0.05)	4.57	17.55

± S.E.

* Significantly different from control at p = 0.05

*** Significantly different from control at p = 0.001.

Table 11b. Effect of boron concentrations on pollen tube groups in S. marginatum

Boron (ppm)	Pollen tubes (%)					
	15-75 (μm)	76-150 (μm)	151-225 (μm)	226-300 (μm)	301-375 (μm)	376-450 (μm)
0	20.00±0.93	30.00±0.45	46.00±0.70	4.00±0.27	0	0
100	13.00±0.49	18.00±0.69	40.00±1.32	22.00±0.66	7.00±0.41	0
200	12.00±0.58	17.00±0.41	43.00±1.42	16.00±0.53	10.00±0.45	2.00±0.16
300	19.00±0.73	17.00±0.32	40.00±1.26	23.00±0.69	1.00±0.12	0
400	27.00±0.94	20.00±0.61	39.00±1.21	13.00±0.67	1.00±0.12	0

± SE

Table 12a. Effect of calcium concentrations on pollen germination and pollen tube growth in *S. marginatum*

Calcium (ppm)	Pollen Germination (%)	Pollen Tube Length (μm)
0	71.49 ± 1.84	266.70 ± 0.35
100	64.28 ± 0.94*	258.00 ± 0.30
200	62.97 ± 2.48**	223.80 ± 0.74**
300	63.48 ± 1.82**	217.20 ± 1.16**
400	62.04 ± 2.32**	216.80 ± 0.49**
LSD (p = 0.05)	5.78	30.45

± S.E.

* Significantly different from control at p = 0.05

** Significantly different from control at p = 0.01.

Table 12b. Effect of calcium concentrations on pollen tube ggoups in S. marginatum

Calcium (ppm)	Pollen tubes (%)					
	15-75 (μm)	76-150 (μm)	151-225 (μm)	226-300 (μm)	301-375 (μm)	376-450 (μm)
0	6.00±0.27	19.00±0.61	7.00±0.34	45.00±1.03	21.00±0.52	2.00±0.40
100	13.00±0.50	12.00±0.47	18.00±0.59	39.00±0.80	18.00±0.47	0
200	20.00±0.68	7.00±0.26	26.00±0.90	32.00±0.79	15.00±0.60	0
300	9.00±0.35	19.00±0.55	31.00±0.95	27.00±0.86	14.00±0.45	0
400	13.00±0.45	17.00±0.50	29.00±0.78	27.00±0.70	14.00±0.52	0

± SE

Table 13a. Effect of magnesium concentrations on pollen germination and pollen tube growth in S. marginatum

Magnesium (ppm)	Pollen Germination (%)	Pollen Tube Length (μm)
0	87.66 ± 0.64	159.90 ± 1.19
100	92.98 ± 0.68**	212.40 ± 1.26***
200	94.35 ± 0.58***	221.70 ± 1.31***
300	92.60 ± 0.64**	218.40 ± 1.23***
400	91.41 ± 0.90*	207.00 ± 1.51**
LSD (p = 0.05)	3.38	28.05

± S.E.

* Significantly different from control at p = 0.05

** Significantly different from control at p = 0.01

*** Significantly different from control at p = 0.001.

Table 13b. Effect of magnesium concentrations on pollen tube groups in S. marginatum

Magnesium (ppm)	Pollen tubes (%)					
	15-75 (μm)	76-150 (μm)	151-225 (μm)	226-300 (μm)	301-375 (μm)	376-450 (μm)
0	46.00±0.49	6.00±0.80	12.00±0.75	24.00±1.20	10.00±1.26	2.00±0.40
100	30.00±0.89	20.00±0.89	2.00±0.40	12.00±0.98	28.00±1.47	8.00±0.74
200	24.00±1.02	22.00±1.16	6.00±0.49	12.00±1.16	16.00±1.62	20.00±1.26
300	22.00±0.74	24.00±0.49	6.00±1.20	12.00±0.98	22.00±1.16	14.00±1.35
400	40.00±1.09	10.00±0.89	4.00±0.49	10.00±1.26	18.00±0.98	18.00±1.33

± SE

Table 14a. Effect of potassium concentrations on pollen germination and pollen tube growth in S. marginatum

Potassium (ppm)	Pollen Germination (%)	Pollen Tube Length (μm)
0	48.46 ± 2.72	117.45 ± 0.79
100	55.50 ± 1.76	124.60 ± 0.99
200	87.46 ± 3.61***	132.00 ± 0.75
300	71.73 ± 1.59***	126.00 ± 0.67
400	59.51 ± 2.20**	120.60 ± 0.71
LSD (p = 0.05)	7.34	19.50

± S.E.

** Significantly different from control at p = 0.01

*** Significantly different from control at p = 0.001.

Table 14b. Effect of potassium concentrations on pollen tube groups in S. marginatum

Potassium (ppm)	Pollen tubes (%)			
	15-75 (μm)	76-150 (μm)	151-225 (μm)	226-300 (μm)
0	50.00±0.00	6.00±1.20	38.00±0.98	6.00±0.49
100	50.00±2.53	24.00±1.55	20.00±0.98	6.00±0.40
200	40.00±1.41	18.00±1.74	30.00±1.36	12.00±0.75
300	40.00±1.09	18.00±1.16	40.00±1.26	2.00±0.40
400	46.00±0.49	24.00±0.80	28.00±0.40	2.00±0.40

± SE

Table 15a. Effect of gelling on pollen germination and pollen tube growth in S. marginatum

Agar (%)	Pollen Germination (%)	Pollen Tube Growth (μm)
0	70.89 ± 2.14	252.30 ± 0.20
0.2	72.86 ± 0.24	235.20 ± 0.63
0.6	72.09 ± 0.97	273.60 ± 0.37*
1.0	75.11 ± 1.02*	296.10 ± 0.43***
1.4	73.75 ± 1.16	259.80 ± 0.46
1.8	68.14 ± 1.65	232.50 ± 0.29*
LSD (p = 0.05)	3.89	18.30

± S.E.

* Significantly different from control at p = 0.05

*** Significantly different from control at p = 0.001.

Table 15b. Effect of gelling on pollen tube groups in S. marginatum

Agar (%)	Pollen tubes (%)					
	15-75 (μm)	76-150 (μm)	151-225 (μm)	226-300 (μm)	301-375 (μm)	376-450 (μm)
0	10.00±0.32	20.00±0.51	30.00±0.32	34.00±0.45	6.00±0.45	0
0.2	6.00±0.22	26.00±0.72	30.00±0.93	34.00±0.57	4.00±0.49	0
0.6	20.00±0.50	2.00±0.40	20.00±0.76	26.00±1.04	10.00±0.40	8.00±0.75
1.0	14.00±0.25	22.00±1.47	12.00±0.32	24.00±0.51	18.00±0.73	10.00±0.89
1.4	20.00±0.93	28.00±0.80	10.00±0.63	26.00±0.80	16.00±0.98	0
1.8	18.00±0.98	24.00±0.60	20.00±0.55	30.00±0.41	8.00±0.75	0

± SE

Table 16a. Effect of pollen germination medium on Pollen germination and pollen tube growth in *S. marginatum*

Medium	Pollen Germination (%)	Pollen Tube Length (μm)
Control[1]	49.90 ± 1.59	179.40 ± 0.75
B & K[2]	59.46 ± 2.51**	209.40 ± 0.98
MB & K[3]	70.49 ± 1.12***	258.00 ± 1.04**
LSD (p = 0.05)	5.65	43.20

± S.E.

** Significantly different from control at p = 0.01

*** Significantly different from control at p = 0.001.

1 Control = Sucrose + Agar

2 B & K = Brewbaker and Kwack's medium (1963)

3 MB & K = Modified Brewbaker and Kwack's medium.

Table 16b. Effect of pollen germination medium on pollen tube groups in S. marginatum

Medium	Pollen tubes (%)			
	15-150 (μm)	151-225 (μm)	226-300 (μm)	301-375 (μm)
Control[1]	39.00±0.89	57.00±0.93	2.00±0.40	2.00±0.40
B & K[2]	44.00±1.39	42.00±1.16	12.00±0.78	2.00±0.40
MB & K[3]	6.00±0.80	46.00±1.16	42.00±0.99	6.00±0.68

± SE

1 Control = Sucrose + Agar

2 B & K = Brewbaker and Kwack's medium (1963)

3 MB & K = Modified Brewbaker and Kwack's medium

Table 17. Effect of growth hormones on pollen germination and pollen tube growth in *S. khasianum*

Growth Hormone	Concentration (ppm)	Pollen Germination (%)	Pollen Tube Length (μm)
Control	0	84.27 ± 1.86	300.00 ± 0.17
Kinetin	1	82.53 ± 1.52	319.50 ± 0.85
	5	69.43 ± 3.77*** (-17.61)	242.10 ± 0.37*** (-19.30)
	10	59.85 ± 3.77*** (-28.98)	114.90 ± 0.31*** (-61.70)
	25	0*** (-100.00)	0*** (-100.00)
IAA	1	82.22 ± 1.21	308.40 ± 0.67
	5	72.84 ± 1.60*** (-13.56)	271.80 ± 0.48* (-9.40)
	10	67.42 ± 3.74*** (-20.00)	185.40 ± 0.74*** (-38.20)
	25	62.38 ± 2.64*** (-25.98)	159.60 ± 0.68*** (-46.80)
GA_3	1	83.33 ± 2.41	301.20 ± 0.28
	5	81.82 ± 2.20	291.00 ± 0.42
	10	80.26 ± 3.47	300.60 ± 0.47
	25	74.89 ± 2.22*** (-11.13)	267.60 ± 0.52** (-10.80)
LSD p=0.05		4.81	22.80

± S.E.

* Significantly different from control at p = 0.05

** Significantly different from control at p = 0.01

*** Significantly different from control at p = 0.001

Figures in parentheses indicate percent inhibition

(-) Over control

Table 18. Effect of growth hormones on Pollen germination and Pollen tube growth in S. marginatum

Growth Hormone	Concentration (ppm)	Pollen Germination (%)	Pollen Tube Length (μm)
Control	0	57.27 ± 1.16	144.45 ± 9.30
Kinetin	1	46.90 ± 0.15*** (-18.11)	86.55 ± 13.65*** (-40.08)
	5	48.01 ± 0.45*** (-16.17)	86.55 ± 10.50*** (-40.08)
	10	37.56 ± 1.92*** (-34.42)	88.50 ± 10.54*** (-38.73)
	25	41.22 ± 3.26*** (-28.03)	82.05 ± 10.54*** (-43.20)
IAA	1	44.14 ± 1.20*** (-22.93)	123.45 ± 12.77
	5	42.36 ± 2.02*** (-26.03)	94.05 ± 8.19*** (-34.89)
	10	39.07 ± 1.48*** (-31.78)	90.45 ± 2.78*** (-37.38)
	25	35.93 ± 2.59*** (-37.26)	73.95 ± 16.46*** (-48.81)
GA_3	1	48.15 ± 1.15*** (-15.92)	124.50 ± 9.76
	5	45.26 ± 1.68*** (-20.97)	126.55 ± 1.80
	10	40.35 ± 0.69*** (-29.54)	125.55 ± 2.65
	25	44.21 ± 0.87*** (-22.80)	126.45 ± 1.00
LSD p=0.05		8.23	25.87

± S.E.

*** Significantly different from control at p = 0.001

Figures in parentheses indicate percent inhibition

(-) Over control

Table 19a. Effects of gamma radiation on pollen germination and pollen tube growth in S. khasianum

Gamma radiation (krad)	Pollen germination (%)	Pollen tube length (μm)
0	74.77±1.87	256.30±0.30
1	70.47±2.02	254.40±0.20
50	72.85±1.40	226.67±0.49* (-11.56)
100	69.82±1.60	215.80±0.89** (-15.80)
200	88.69±3.30*** (+18.62)	214.05±1.08** (-16.48)
500	55.93±1.39*** (-28.59)	88.95±1.35*** (-63.14)
600	52.30±2.77*** (-30.05)	75.71±0.07*** (-70.46)
700	28.78±1.94*** (-61.51)	31.37±0.17*** (-87.76)
800	0 (-100.00)***	0 (-100.00)***
LSD p=0.5	6.89	25.05

± S.E.

* Significantly different from control at p=0.05

** Significantly different from control at p=0.01

*** Significantly different from control at p=0.001.

Figures in parentheses indicate percent stimulation (+)/inhibition(-) over control.

Table 19b. Effect of gamma radiation on pollen tube groups in S. khasianum

Gamma radiation (krad)	Pollen tubes (%)				
	15-75 (μm)	76-150 (μm)	151-225 (μm)	226-300 (μm)	301-375 (μm)
0	0	0	42.00±1.47	56.00±1.62	2.00±0.40
1	0	0	18.00±0.75	76.00±1.20	6.00±0.80
50	0	4.00±0.49	52.00±1.47	44.00±1.62	0
100	0	6.00±0.80	62.00±2.13	32.00±2.75	0
200	0	2.00±0.40	72.00±3.25	26.00±3.38	0
500	40.00±2.76	58.00±2.48	2.00±0.40	0	0
600	92.00±0.49	8.00±0.49	0	0	0
700	98.00±0.70	2.00±0.40	0	0	0
800	0	0	0	0	0

± S.E.

Table 20. Effect of growth hormones and modulation of gamma radiation (500 krad) responses of pollen germination and pollen tube growth in S. khasianum

	Treatment	Control	Kinetin (ppm)				IAA (ppm)				GA_3 (ppm)			
			1	5	10	25	1	5	10	25	1	5	10	25
Pollen Germination (%)	Unirradiated	65.89 ±1.47	60.72 ±1.47	47.62*** ±3.77 (-27.73)	38.04*** ±3.77 (-42.27)	0*** (-100.00)	60.41 ±1.21	51.03*** ±1.60 (-22.55)	45.61*** ±3.74 (-30.78)	40.57*** ±2.64 (-38.43)	61.52 ±2.41	60.01 ±2.20	60.50 ±3.47	55.08*** ±2.22 (-19.44)
	Irradiated	37.50*** ±4.91 (-43.09)	32.81 ±1.00	33.87 ±4.37	21.86*** ±1.65 (-41.71)	0*** (-100.00)	37.09 ±0.89	37.67 ±1.52	28.86** ±0.84 (-23.04)	25.36** ±2.24 (-32.37)	38.37 ±2.81	47.50** ±2.28 (+26.67)	50.80*** ±3.65 (+35.47)	39.49 ±2.44
Pollen Tube Length (µm)	Unirrated	254.40 ±0.50	261.34 ±0.85	214.90*** ±0.31 (-15.53)	107.04*** ±0.31 (-57.92)	0*** (-100.00)	251.92 ±0.67	240.09* ±0.48 (-5.63)	166.82*** ±0.74 (-34.43)	144.94*** ±0.68 (-43.03)	255.42 ±0.28	246.77 ±0.42	254.91 ±0.47	232.52*** ±0.32 (-7.05)
	Irradiated	79.20*** ±0.40 (-68.87)	60.60** ±0.42 (-23.48)	41.40*** ±0.21 (-47.73)	27.50*** ±0.18 (-65.55)	0*** (-100.00)	48.30*** ±0.05 (-39.02)	46.50*** ±0.32 (-41.29)	33.30*** ±0.45 (-57.95)	25.80*** ±0.45 (-67.42)	86.70 ±0.26	79.80 ±0.30	80.10 ±0.21	70.20 ±0.14

± S.E.

* Significantly different from control at p=0.05

** Significantly different from control at p=0.01

*** Significantly different from control at p=0.001.

Figures in parenthesis indicate percent stimulation (+)/inhibition (-) over the respective control.

Table 21. Effects of growth hormones and gamma radiation (600 krad) on cytochemical constituents of S. khasianum pollen tubes

Growth hormones (10 ppm)	Un-irradiated									Irradiated								
	Insoluble polysaccharides			Protein			Nucleic acid (RNA)			Insoluble polysaccharides			Protein			Nucleic acids (RNA)		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Control	+++	++	++	+++	+++	+++	++	++	++	++++	++++	++++	++++	++++	++++	+	++	+
Kinetin	+++	+++	+++	++++	++++	++++	++	++	++	++++	++++	++++	++++	++++	++++	++	++	++
IAA	+++	+++	+++	++	++	++	+	++	++	++++	++++	++++	++++	++++	++++	+	++	+
GA_3	+++	+++	++	++	++	++	++	++	++	++++	++++	++++	++++	++++	++++	++	++	+

1. Apical region
2. Sub-apical region
3. Distal region

\+ Faint
++ Moderate
+++ High
++++ Very high

Table 22a. Effect of gamma radiation on pollen germinationa and pollen tube growth in S. marginatum

Gamma radiation (krad)	Pollen germination (%)	Pollen tube length (μm)
0	64.31±0.22	299.55±0.25
1	61.61±0.22	294.50±0.50
50	63.63±0.32	289.60±0.90
100	66.43±0.75	288.60±1.07
200	76.92±0.35*** (+19.61)	259.05±0.37*** (-13.52)
500	36.36±1.41 (-43.46)	95.55±0.43*** (-68.10)
600	24.48±1.60*** (-61.93)	83.10±0.14*** (-72.26)
700	19.69±0.96*** (-69.38)	51.60±0.18*** (-82.77)
800	0 (-100.00)***	0 (-100.00)***
LSD p=0.05	7.60	25.35

± S.E.

*** Significantly different from control at p=0.001. Figures in parentheses indicate percent stimulation (+)/inhibition (-) over control.

Table 22b. Effect of gamma irradiation on pollen tube groups in S. marginatum

Gamma radiation (krad)	Pollen tubes (%)					
	15-75 (μm)	76-150 (μm)	151-225 (μm)	226-300 (μm)	301-375 (μm)	376-450 (μm)
0	0	0	12.00±3.74	44.00±1.67	26.00±8.72	18.00±4.00
1	0	8.00±2.00	14.00±2.45	44.00±7.48	24.00±5.10	10.00±3.16
50	0	8.00±3.74	26.00±8.72	46.00±6.78	20.00±7.07	0
100	0	12.00±5.83	20.00±4.47	50.00±10.00	18.00±9.17	0
200	0	3.00±2.11	35.00±1.23	62.00±1.25	0	0
500	53.00±7.15	30.00±8.16	17.00±4.94	0	0	0
600	48.00±4.90	52.00±4.90	0	0	0	0
700	88.00±3.74	12.00±3.74	0	0	0	0
800	0	0	0	0	0	0

± S.E.

Table 23. Effect of growth hormones and modulation of gamma radiation (500 krad) responses of pollen germination and pollen tube growth in S. marginatum

	Treatment	Control	Kinetine (ppm)				IAA (ppm)				GA_3 (ppm)			
			1	5	10	25	1	5	10	25	1	5	10	25
Pollen Germination (%)	Unirradiated	81.46 ±1.43	71.09*** ±0.16 (-12.73)	72.20*** ±0.15 (-11.37)	65.41*** ±0.92 (-19.70)	61.75*** ±0.26 (-24.20)	68.33*** ±0.20 (-16.12)	66.55*** ±0.25 (-18.30)	63.26*** ±0.48 (-22.34)	60.12*** ±0.57 (-26.20)	72.54*** ±0.25 (-11.20)	69.45*** ±0.65 (-14.74)	68.40*** ±0.69 (-16/03)	64.54*** (-20.77)
	Irradiated	57.49 ±1.39 (-55.98)	37.75 ±2.18	35.82 ±0.90	32.28* ±1.82 (-13.90)	26.54*** ±0.99 (-29.74)	41.14 ±0.48	39.70 ±0.52	32.33* ±0.14 (-15.76)	27.72*** ±0.28 (-26.06)	35.24 ±1.20	33.40 ±0.92	35.45 ±1.28	35.00 ±1.51
Pollen tube length (μm)	Unirradiated	305.70 ±0.70	247.80*** ±3.65 (-18.94)	246.75*** ±2.50 (-19.28)	248.75*** ±2.54 (-18.63)	243.30*** ±1.61 (-20.41)	285.70 ±2.73	255.30*** ±1.19 (-16.49)	251.70*** ±1.75 (-17.66)	235.20*** ±1.46 (-23.06)	285.75 ±1.76	286.80 ±1.80	287.75 ±1.65	286.65 ±1.15
	Irradiated	150.90 ±0.22 (-50.64)	131.50 ±0.30	115.70** ±0.31 (-23.33)	110.80*** ±0.35 (-26.57)	70.20*** ±0.18 (-53.48)	135.90 ±0.42	75.30*** ±0.52 (-50.10)	72.30*** ±0.14 (-52.09)	66.60*** ±0.28 (-55.86)	165.60 ±1.00	157.80 ±0.54	158.70 ±0.65	149.70 ±0.38

± S.E.

* Significantly different from control at p=0.05

** Significantly different from control at p=0.01

*** Significantly different from control at p=0.001

Figures in parenthesis indicate percent stimulation (+)/inhibition (-) over the respective control.

Table 24. Effects of growth hormones and gamma radiation (500 krad) on cytochemical constituents of S. marginatum pollen tubes

Growth hormones (10 ppm)	Un-irradiated									Irradiated								
	Insoluble polysaccharides			Protein			Nucleic acids (RNA)			Insoluble polysaccharides			Protein			Nucleic acids (RNA)		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Control	+	+	+	++	++	++	++	++	++	+	++	++	++++	++++	++++	++	++	++
Kinetin	++	++	++	+++	+++	+++	++	++	++	+++	+++	+++	++++	++++	++++	+	+	+
IAA	++	++	++	++	++	++	++	+++	++	+++	+++	+++	++	+++	+++	+	+	+
GA_3	+	+	+	++	++	++	++	+++	+++	+++	+++	+++	++	++	++	++	++	++

1. Apical region
2 Sub-apical region
3 Distal region

\+ Faint
++ Moderate
+++ High
++++ Very high

Fig. 1 : Solanum khasianum plant with berries (be).

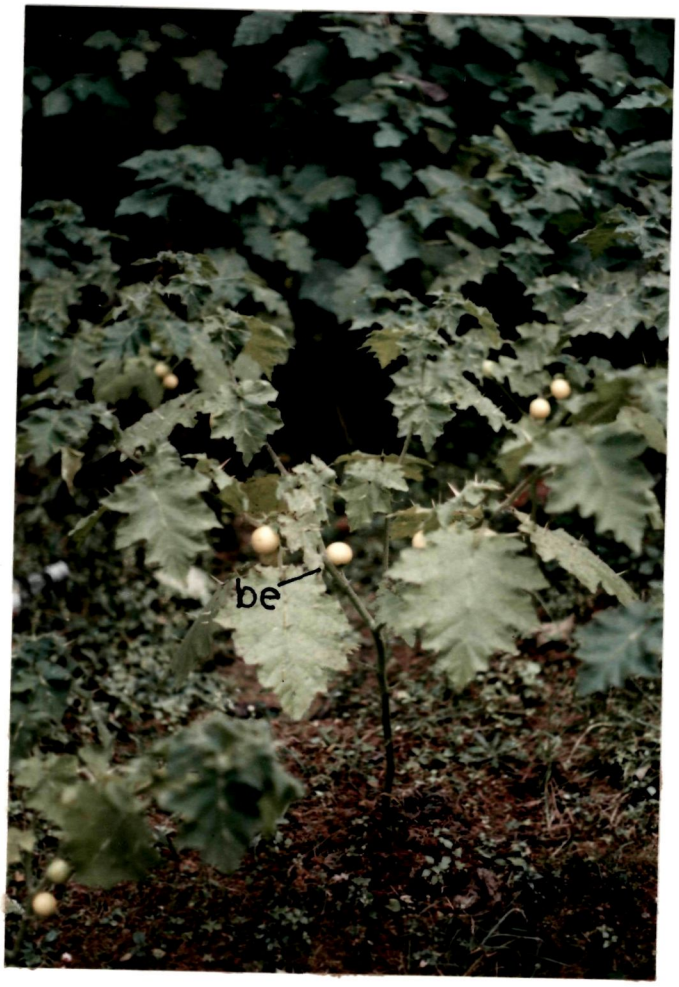


Fig.1

Fig. 2 : Effect of pH on S. khasianum pollen germination and pollen tube elongation.

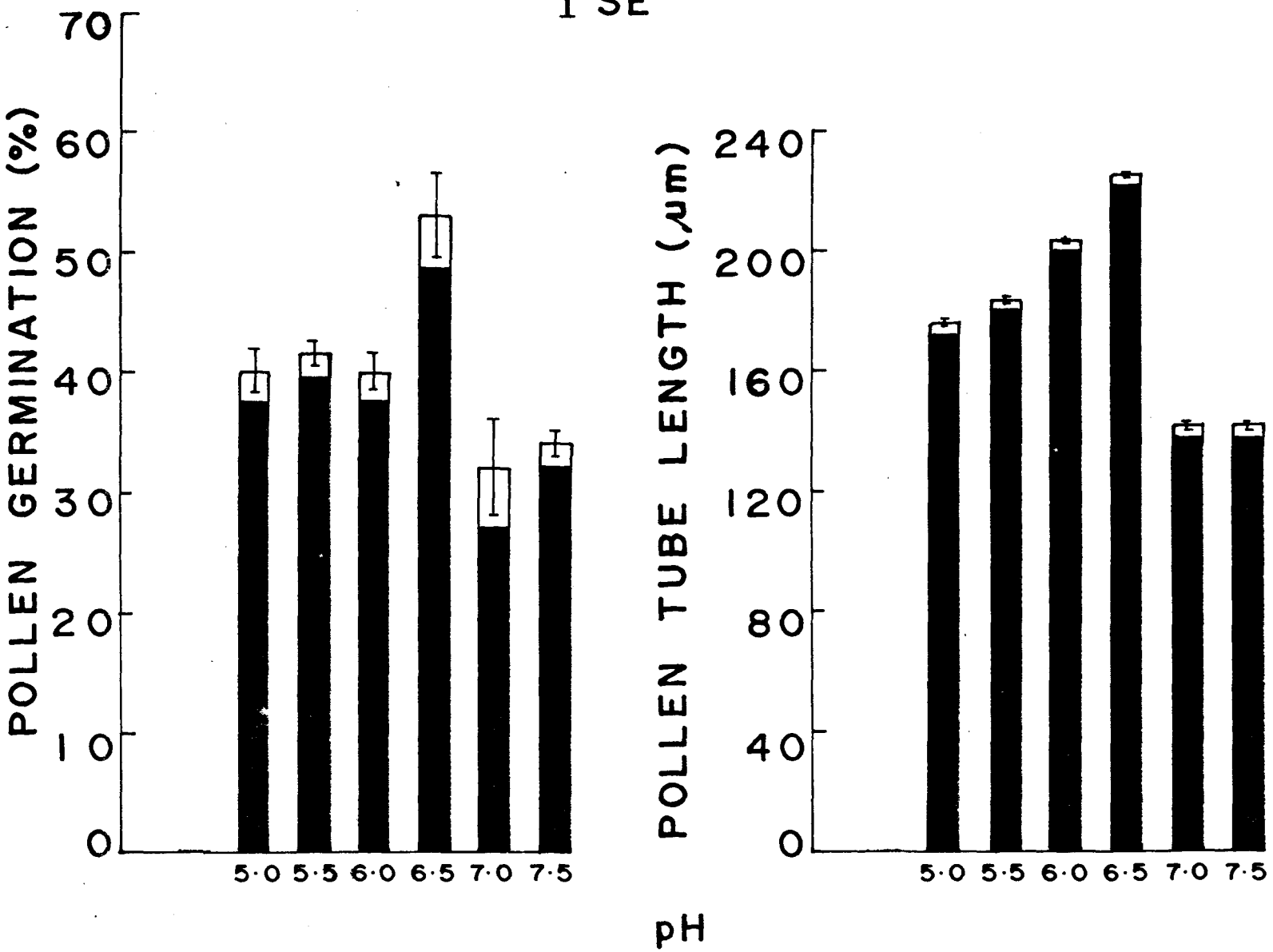

I SE
POLLEN GERMINATION (%)
70
60
50
40
30
20
10
0
5·0 5·5 6·0 6·5 7·0 7·5
POLLEN TUBE LENGTH (μm)
240
200
160
120
80
40
0
5·0 5·5 6·0 6·5 7·0 7·5
pH

Fig.2

Fig. 3a : Effect of Calcium on S. khasianum pollen germination.

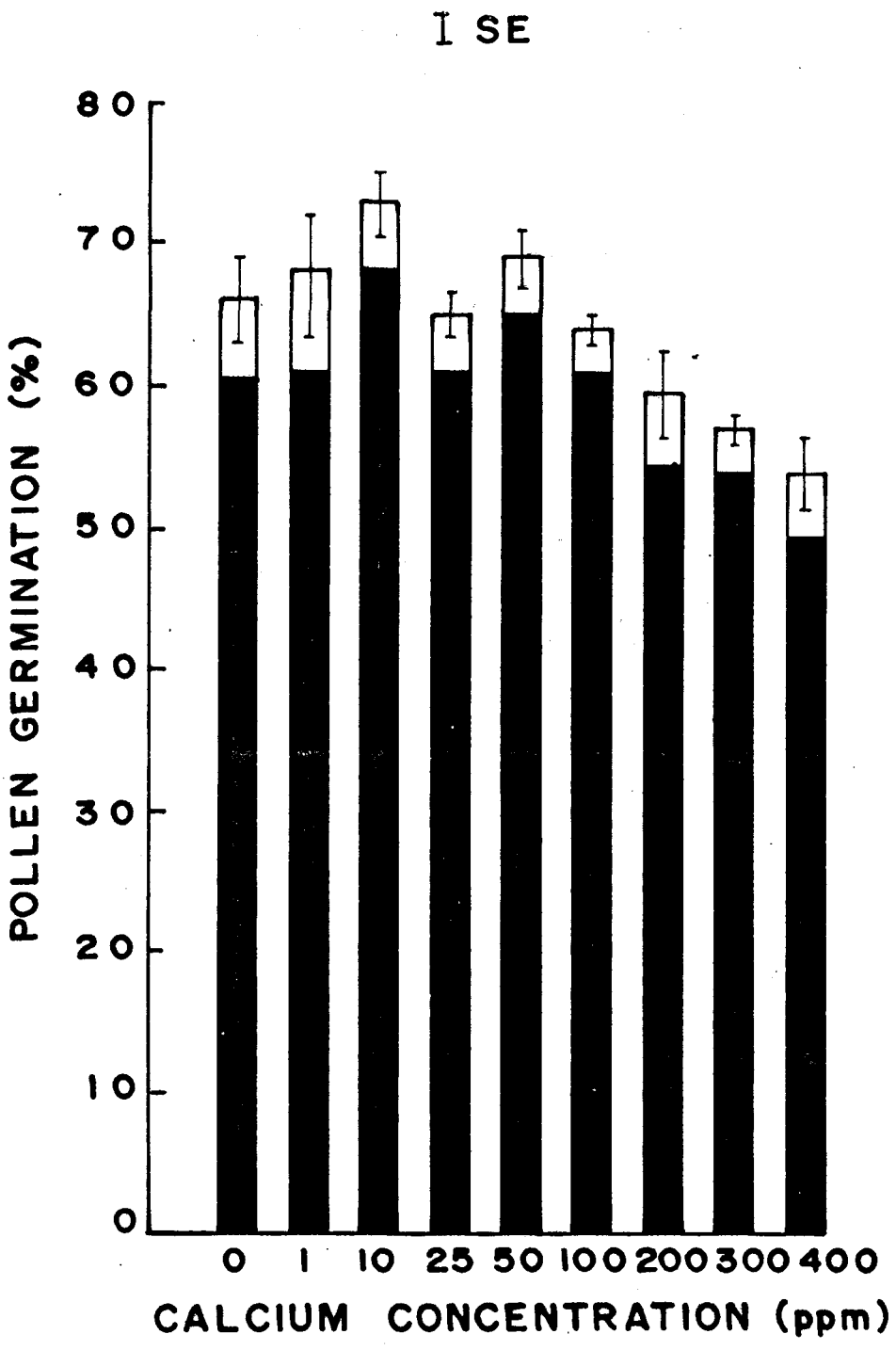

I SE
80
70
60
50
40
30
20
10
0
POLLEN GERMINATION (%)
0 1 10 25 50 100 200 300 400
CALCIUM CONCENTRATION (ppm)

Fig. 3a

Fig. 3b : Effect of Calcium on S. khasianum pollen tube elongation.

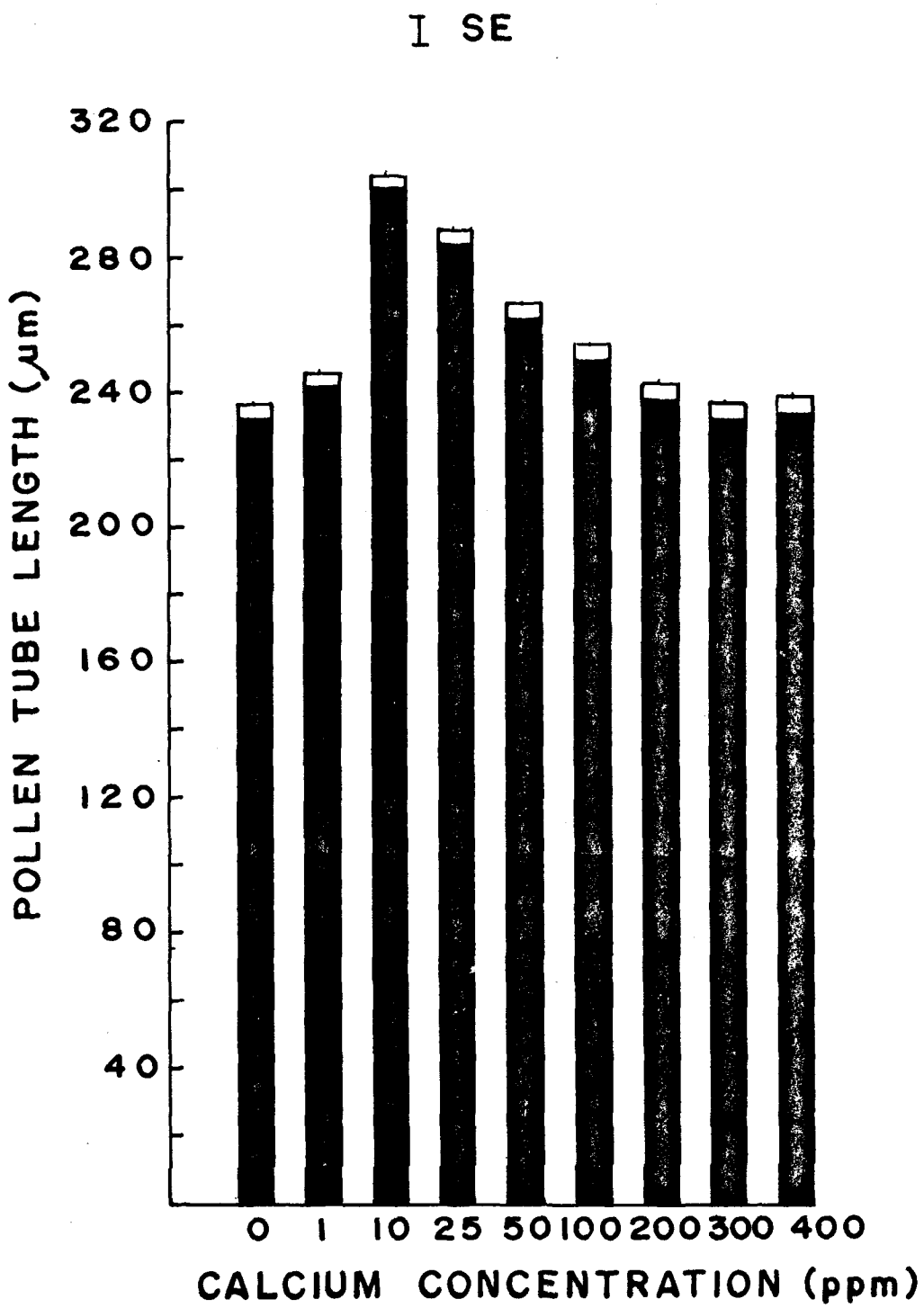

I SE
320
280
240
200
160
120
80
40
POLLEN TUBE LENGTH (μm)
0 1 10 25 50 100 200 300 400
CALCIUM CONCENTRATION (ppm)

Fig.3b

Fig. 4 : Effect of Magnesium on S. khasianum pollen germination and pollen tube elongation.

ISE

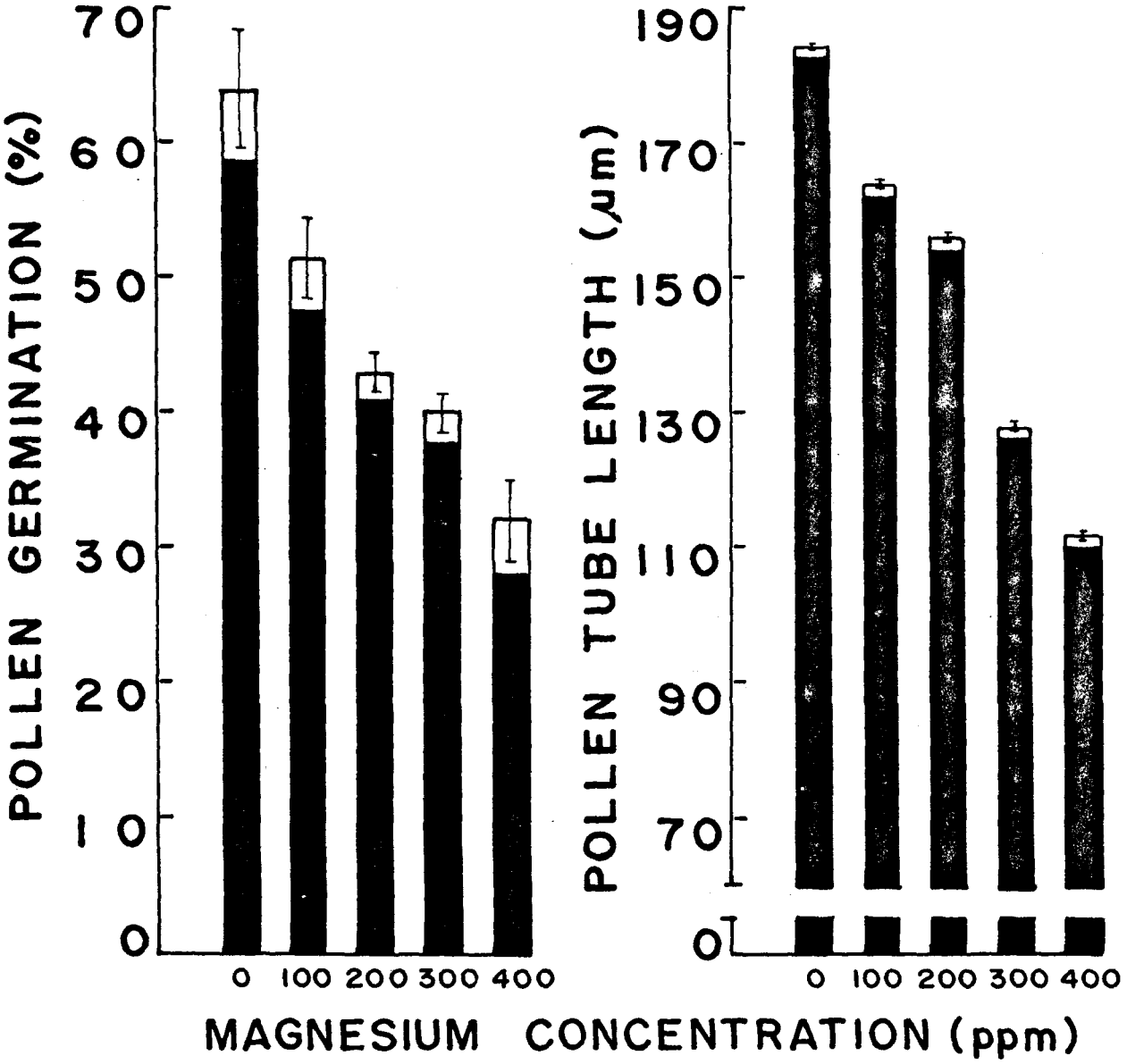

POLLEN GERMINATION (%)
70
60
50
40
30
20
10
0
0 100 200 300 400
POLLEN TUBE LENGTH (μm)
190
170
150
130
110
90
70
0
0 100 200 300 400
MAGNESIUM CONCENTRATION (ppm)

Fig.4

Fig. 5 : Effect of Potassium on S. khasianum pollen germination and pollen tube elongation.

ISE

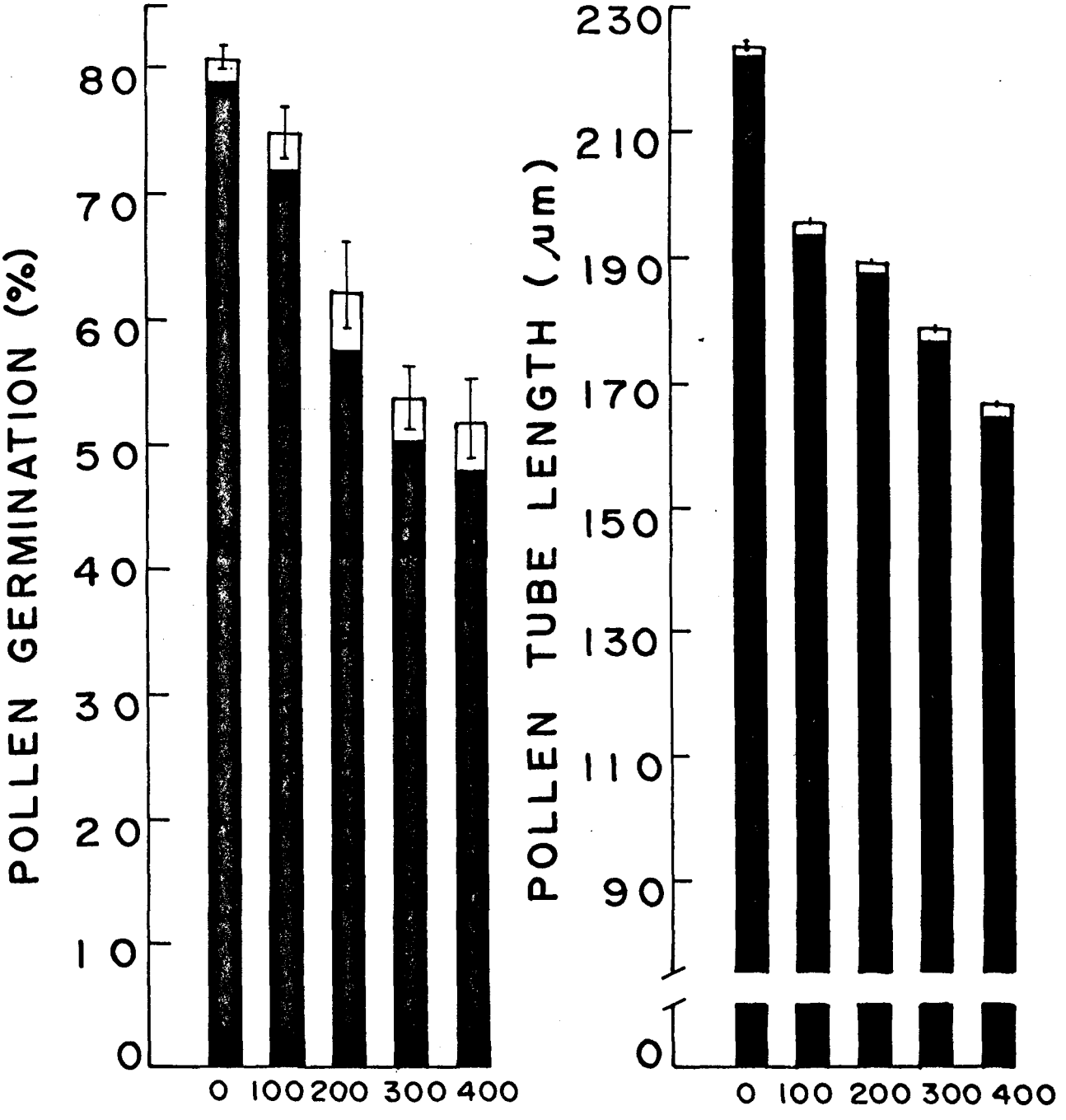

POLLEN GERMINATION (%)
80
70
60
50
40
30
20
10
0
0 100 200 300 400
POLLEN TUBE LENGTH (μm)
230
210
190
170
150
130
110
90
0
0 100 200 300 400
POTASSIUM CONCENTRATION (ppm)

Fig.5

Fig. 6 : Effect of gelling on S. khasianum pollen germination and pollen tube elongation.

I SE

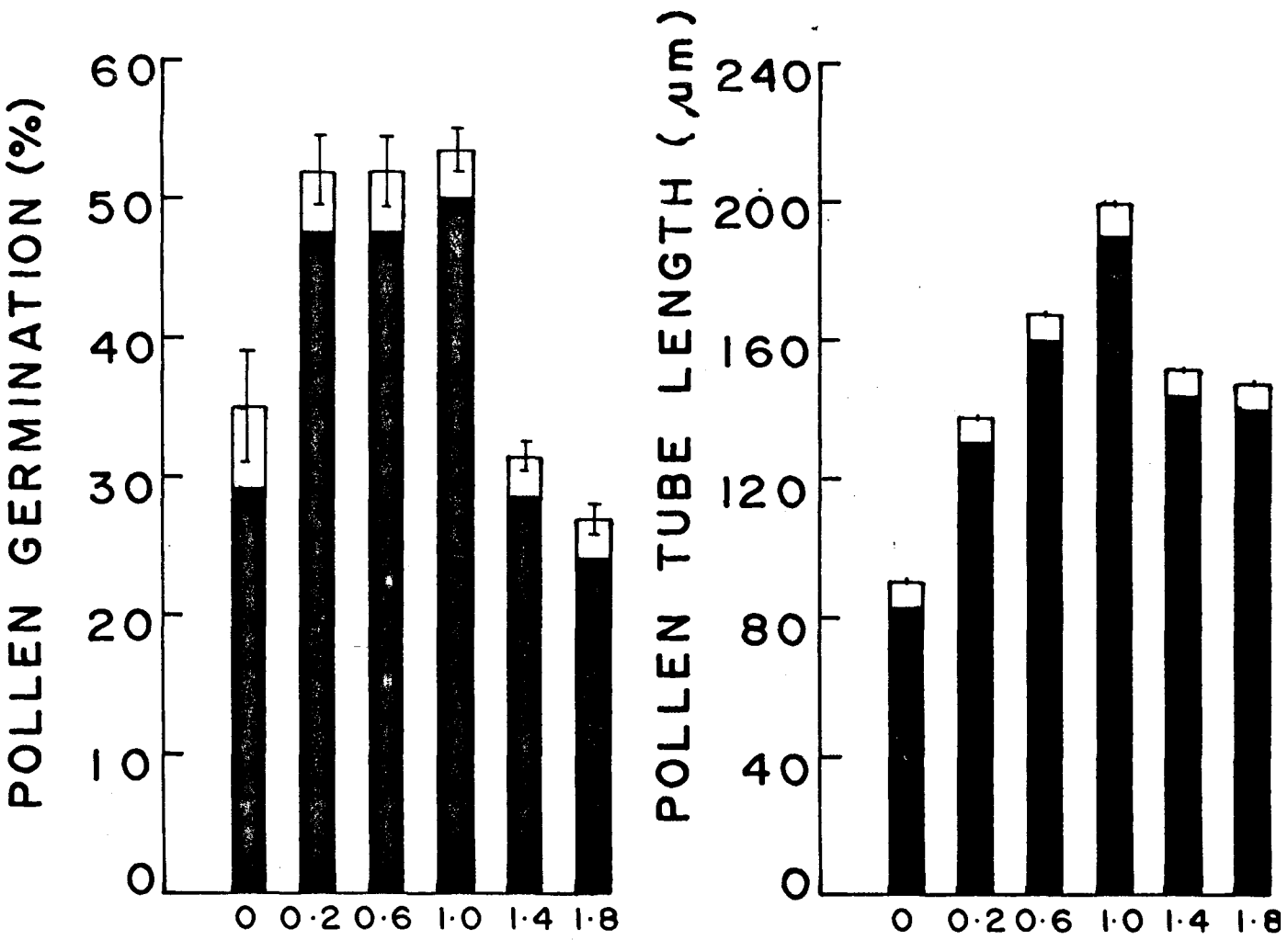
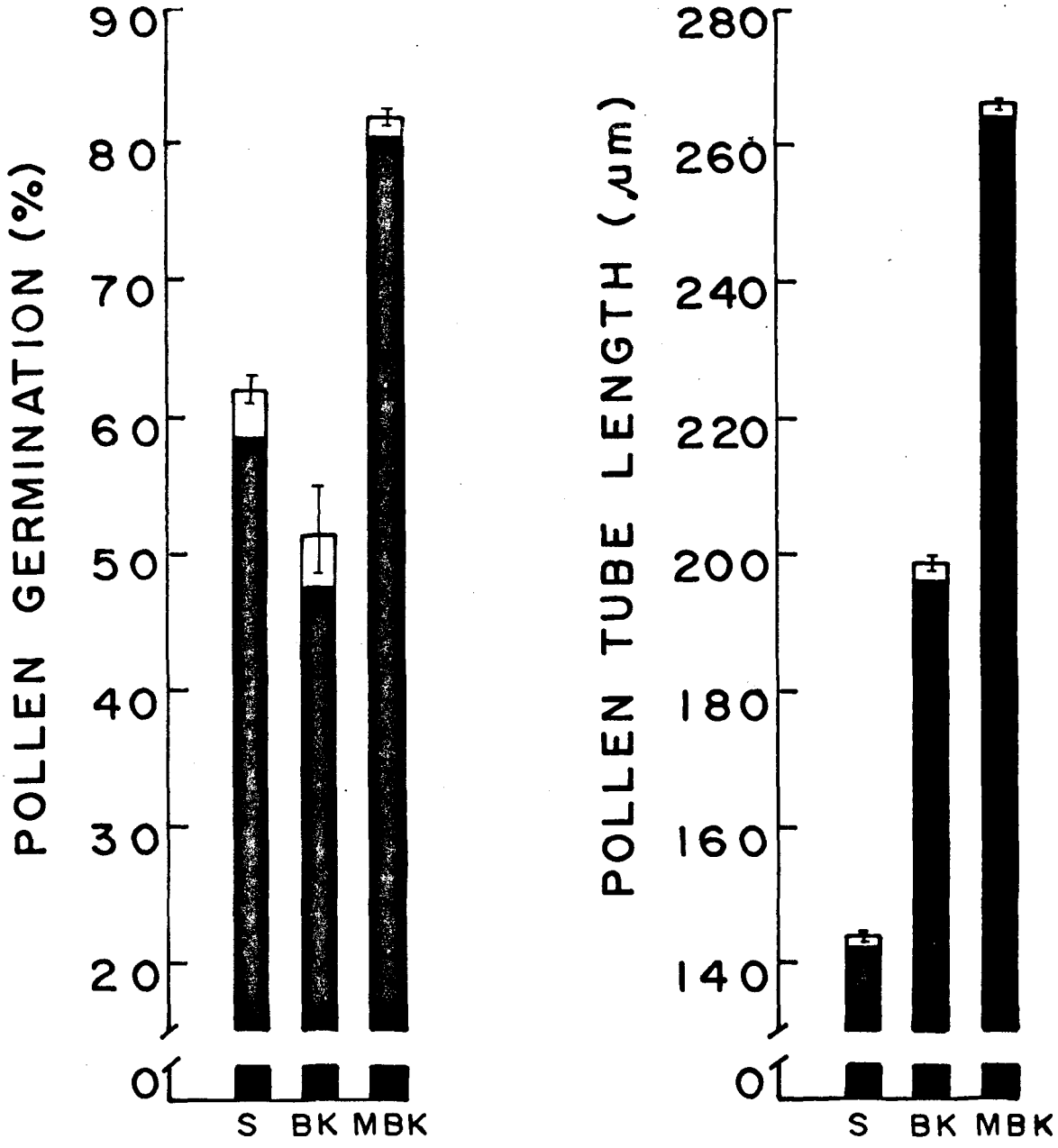

POLLEN GERMINATION (%)
60
50
40
30
20
10
0
0 0·2 0·6 1·0 1·4 1·8
POLLEN TUBE LENGTH (μm)
240
200
160
120
80
40
0
0 0·2 0·6 1·0 1·4 1·8
AGAR CONCENTRATION (ppm)

Fig.6

Fig. 7 : Effect of different pollen germination media on *S. khasianum* pollen germination and pollen tube elongation.

ISE

S=SUCROSE 15%
BK=BREWBAKER & KWACK'S (1963) MEDIUM
MBK=MODIFIED BREWBAKER & KWACK MEDIUM



MEDIUM

Fig. 7

Fig. 8 : Solanum marginatum plant.

Fig.8

Fig. 9 : Effect of temperature on S. marginatum pollen germination and pollen tube elongation.

I SE

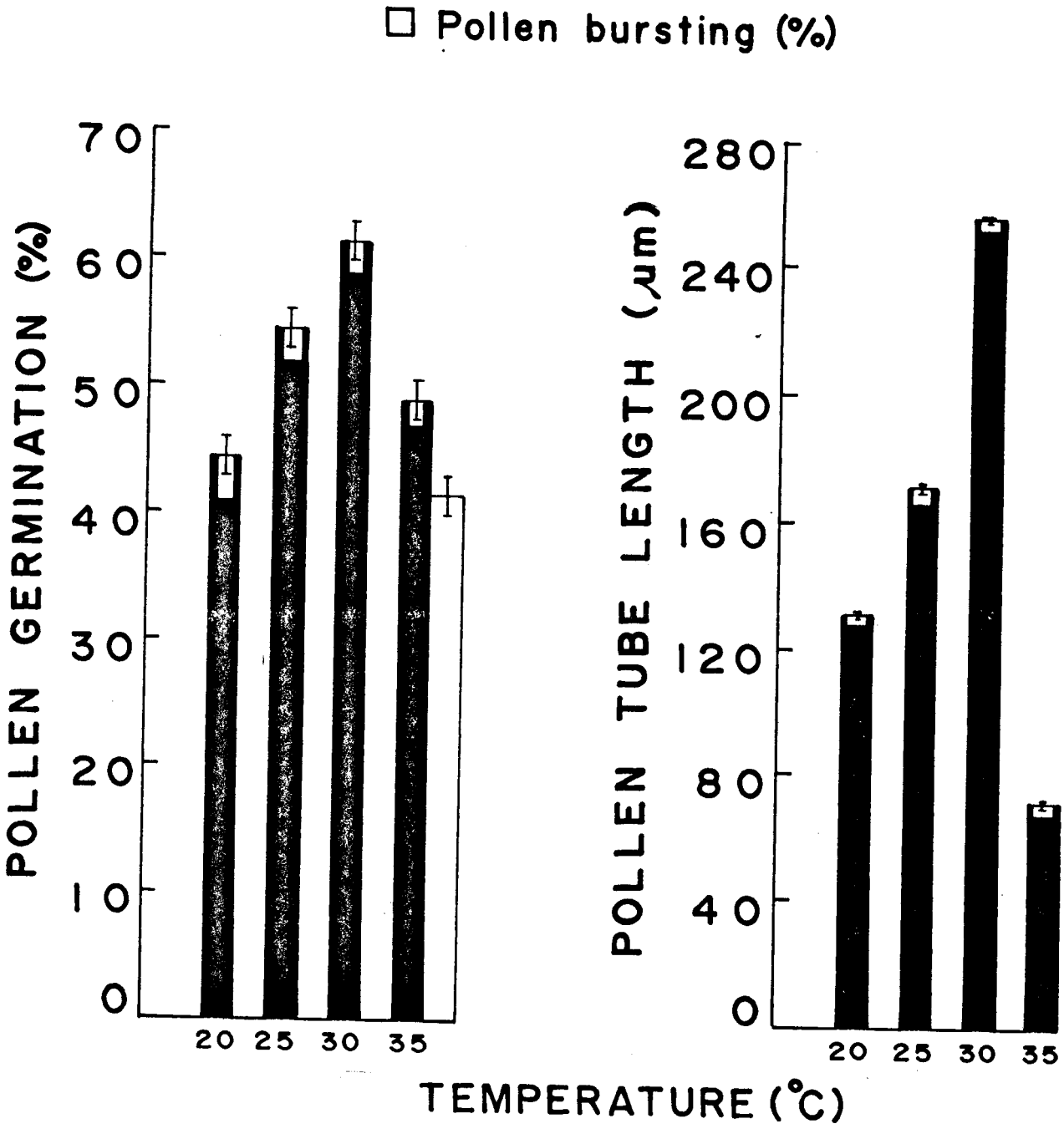

□ Pollen bursting (%)
POLLEN GERMINATION (%)
0
10
20
30
40
50
60
70
20 25 30 35
POLLEN TUBE LENGTH (μm)
0
40
80
120
160
200
240
280
20 25 30 35
TEMPERATURE (°C)

Fig.9

Fig. 10 : Effect of pH on *S. marginatum* pollen germination and pollen tube elongation.

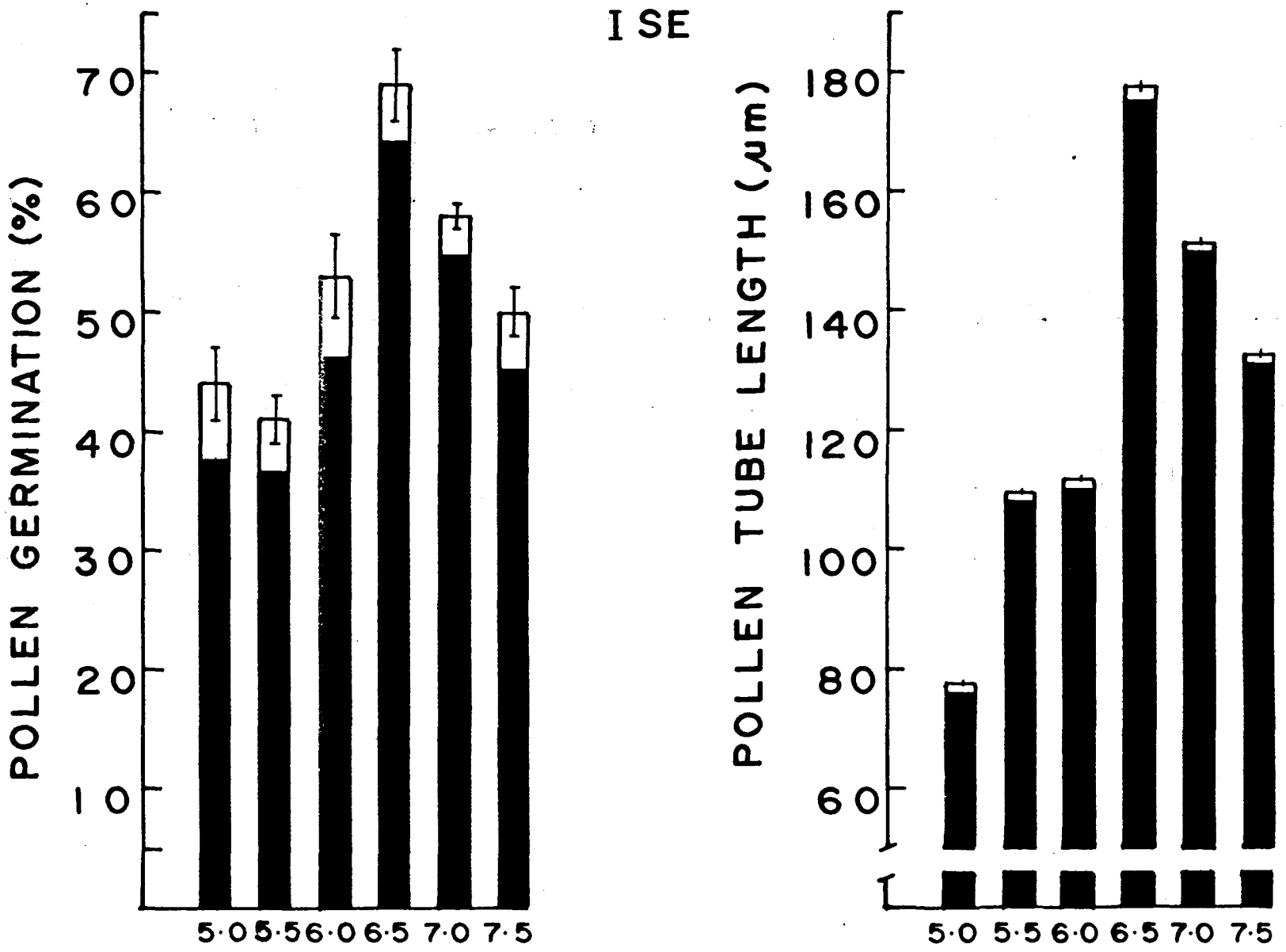

I SE
POLLEN GERMINATION (%)
70
60
50
40
30
20
10
5·0 5·5 6·0 6·5 7·0 7·5
POLLEN TUBE LENGTH (μm)
180
160
140
120
100
80
60
5·0 5·5 6·0 6·5 7·0 7·5
pH

Fig. 10

Fig. 11 : Effect of Sucrose on S. marginatum pollen germination and pollen tube elongation.

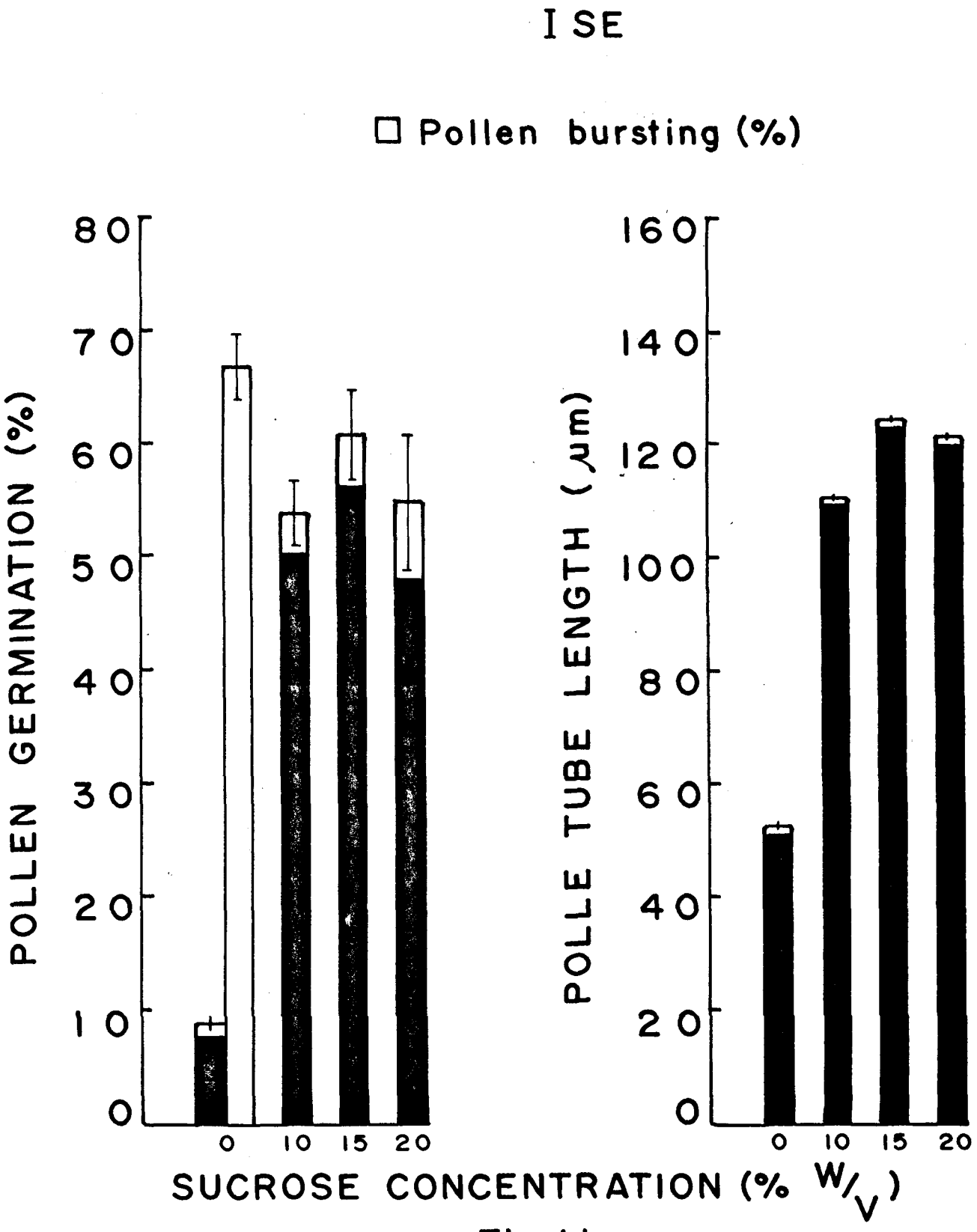

I SE
□ Pollen bursting (%)
POLLEN GERMINATION (%)
80
70
60
50
40
30
20
10
0
POLLE TUBE LENGTH (μm)
160
140
120
100
80
60
40
20
0
0 10 15 20
0 10 15 20
SUCROSE CONCENTRATION (% W/V)

Fig.11

Fig. 12 : Effect of Boron on *S. marginatum* pollen germination and pollen tube elongation.

I SE

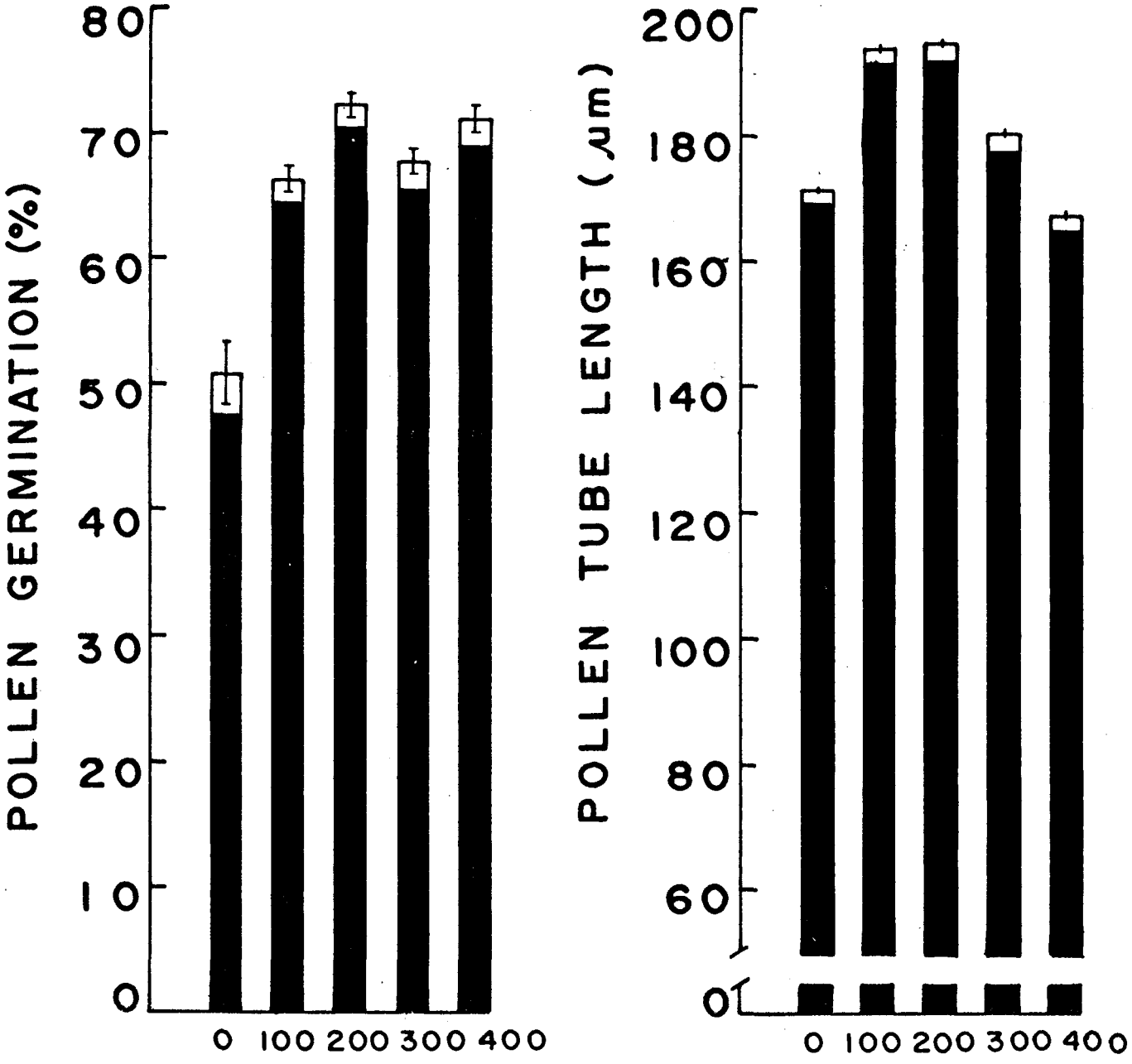

POLLEN GERMINATION (%)
80
70
60
50
40
30
20
10
0
0 100 200 300 400
POLLEN TUBE LENGTH (μm)
200
180
160
140
120
100
80
60
0
0 100 200 300 400
BORON CONCENTRATION (ppm)

Fig.12

Fig. 13 : Effect of Calcium on S. marginatum pollen germination and pollen tube elongation.

I SE

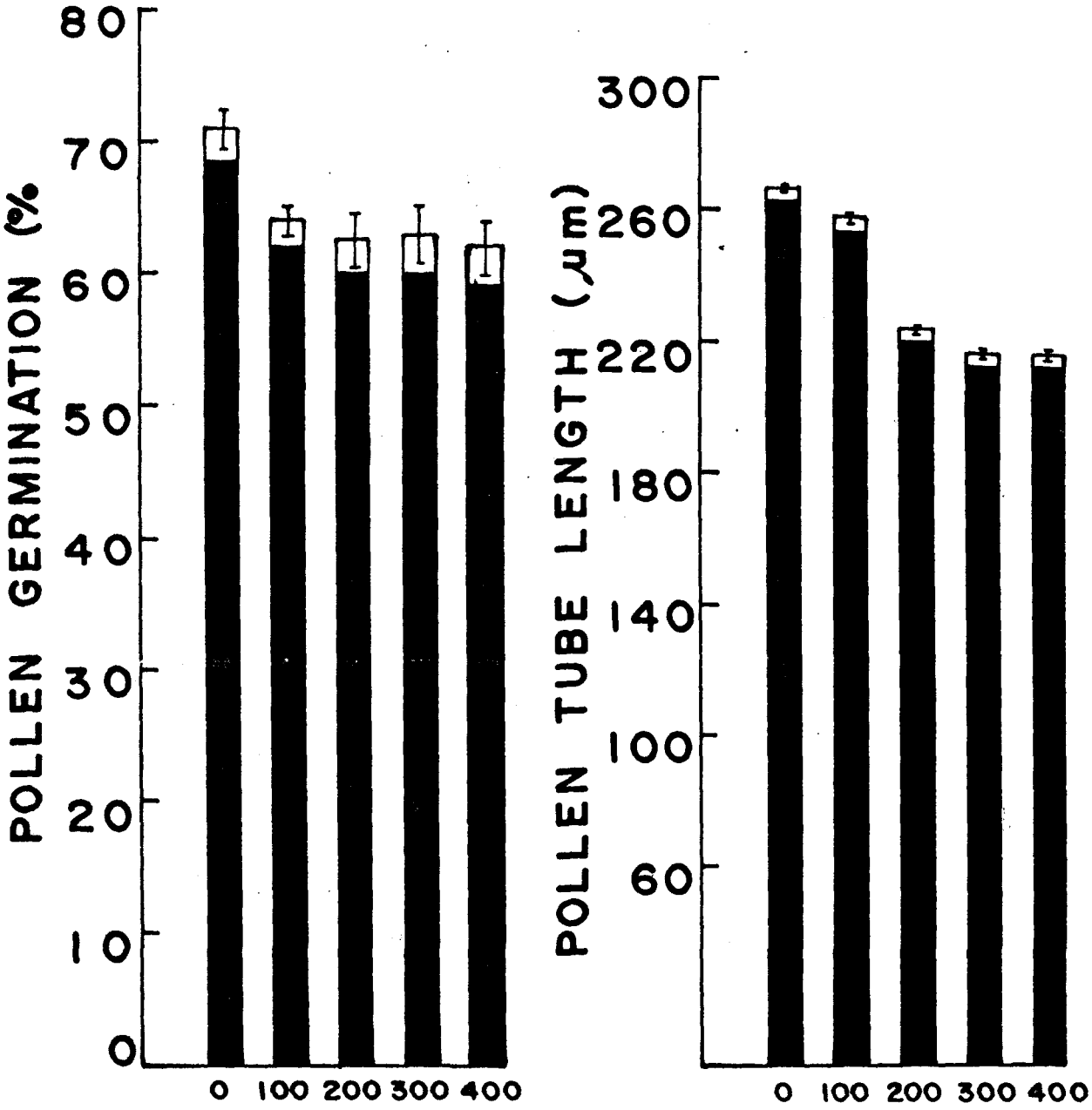

POLLEN GERMINATION (%)
80
70
60
50
40
30
20
10
0
0 100 200 300 400
POLLEN TUBE LENGTH (μm)
300
260
220
180
140
100
60
0 100 200 300 400
CALCIUM CONCENTRATION (ppm)

Fig.13

Fig. 14 : Effect of Magnesium on S. marginatum pollen germination and pollen tube elongation.

I SE

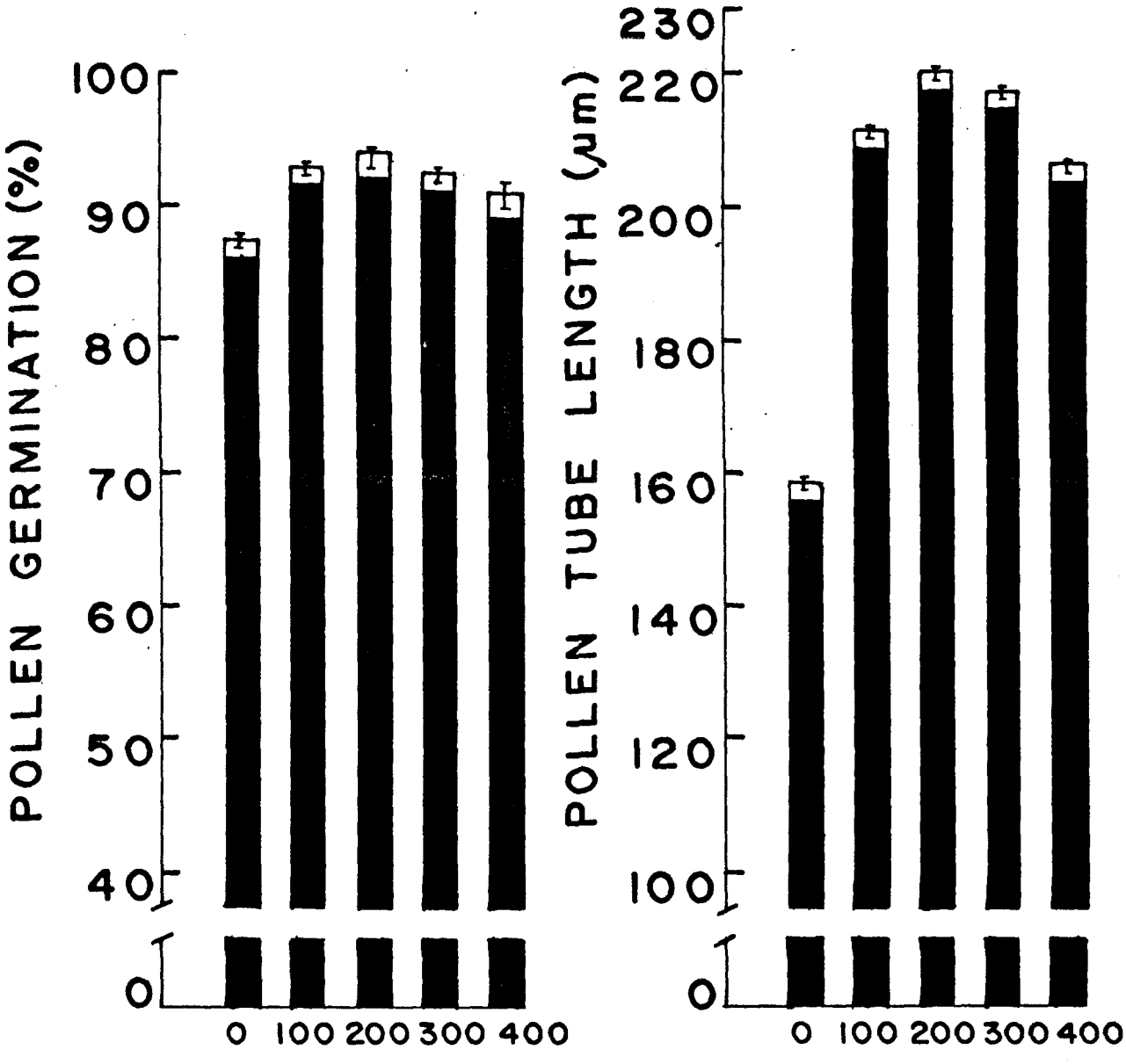

POLLEN GERMINATION (%)
100
90
80
70
60
50
40
0
0 100 200 300 400
POLLEN TUBE LENGTH (μm)
230
220
200
180
160
140
120
100
0
0 100 200 300 400
MAGNESIUM CONCENTRATION (ppm)

Fig.14

Fig. 15 : Effect of Potassium on S. marginatum pollen germination and pollen tube elongation.

ISE

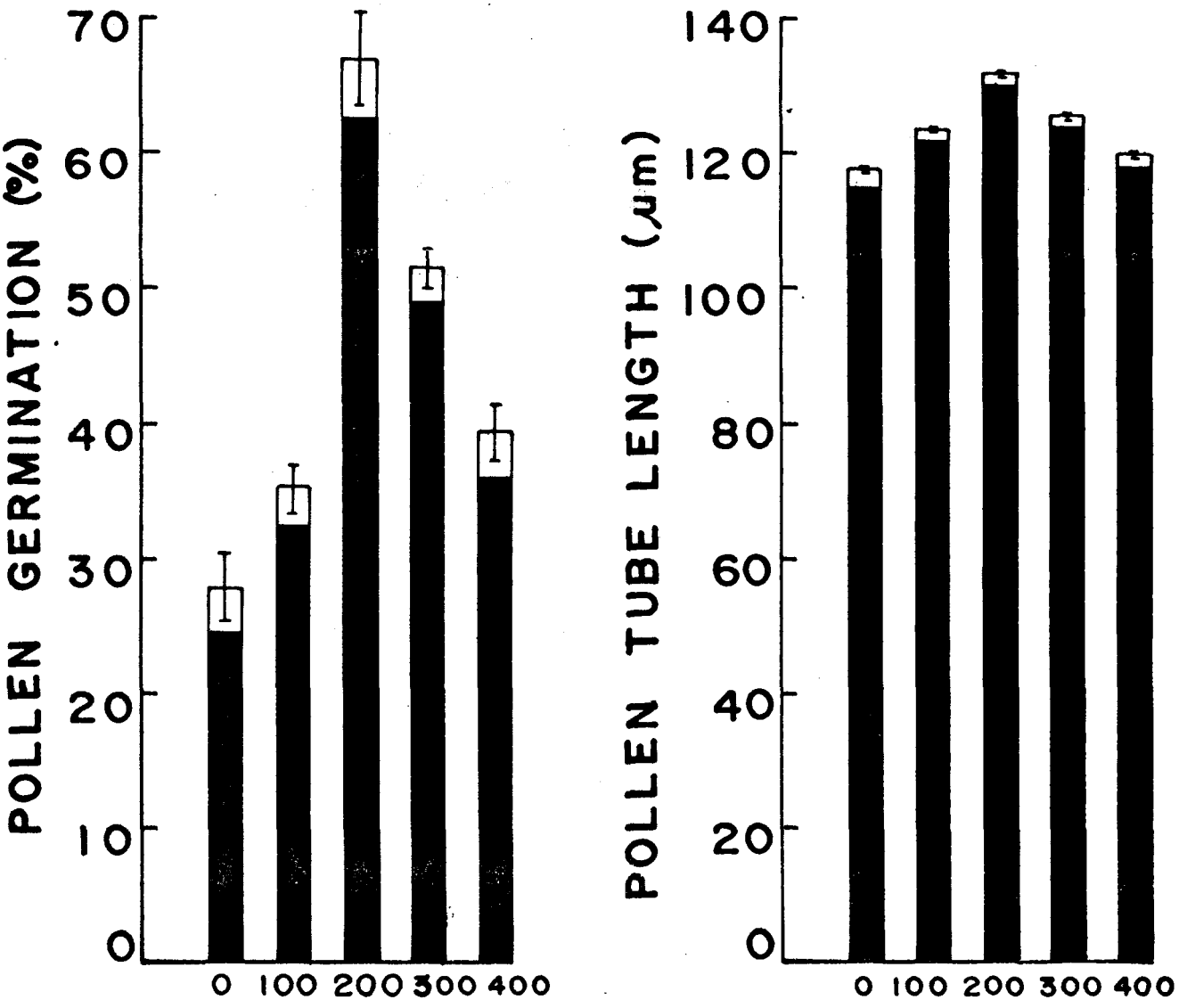

POLLEN GERMINATION (%)
0
10
20
30
40
50
60
70
POLLEN TUBE LENGTH (μm)
0
20
40
60
80
100
120
140
0 100 200 300 400
0 100 200 300 400
POTASSIUM CONCENTRATION (ppm)

Fig.15

Fig. 16 : Effect of gelling on S. marginatum pollen germination and pollen tube elongation.

I SE

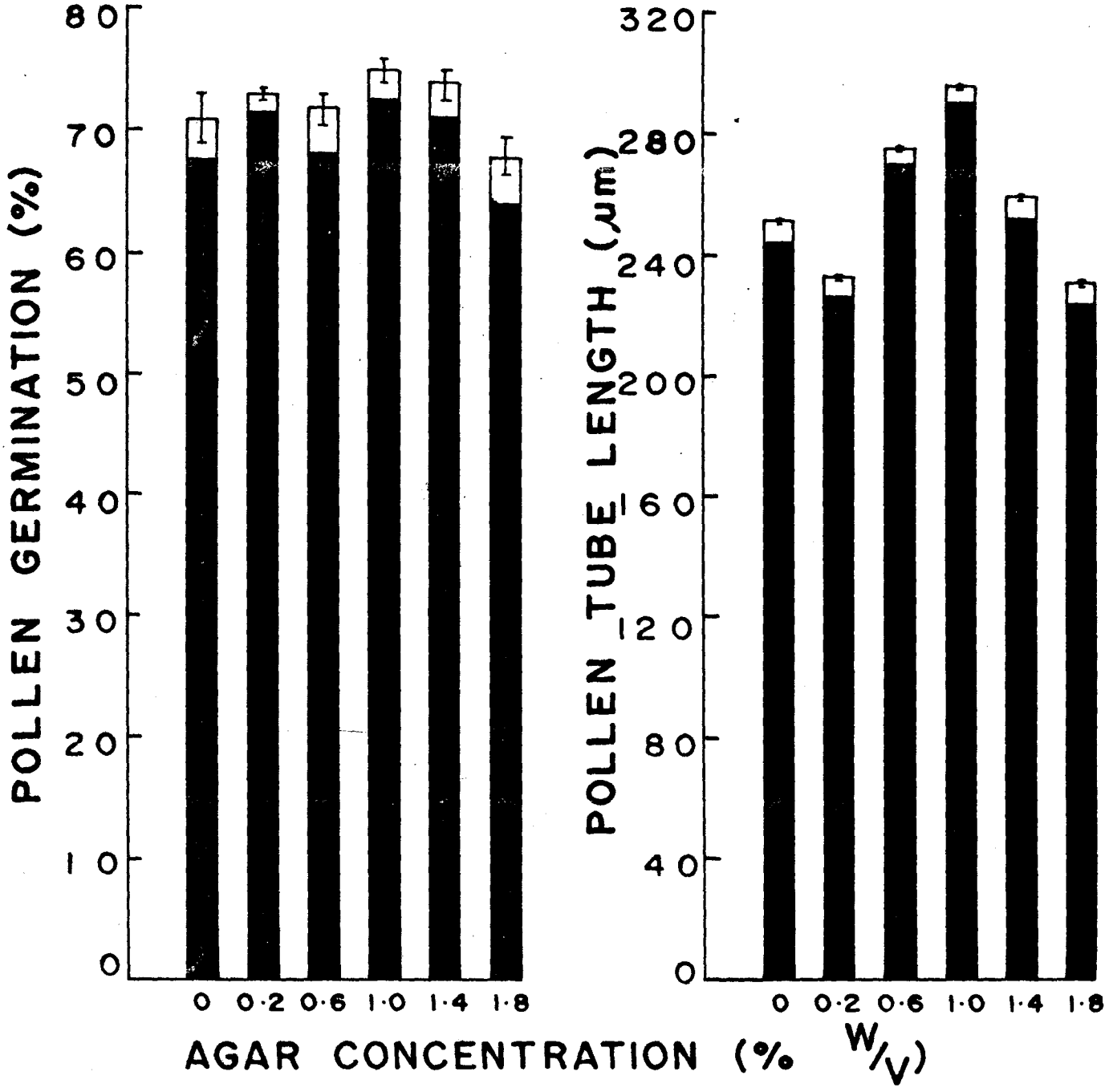
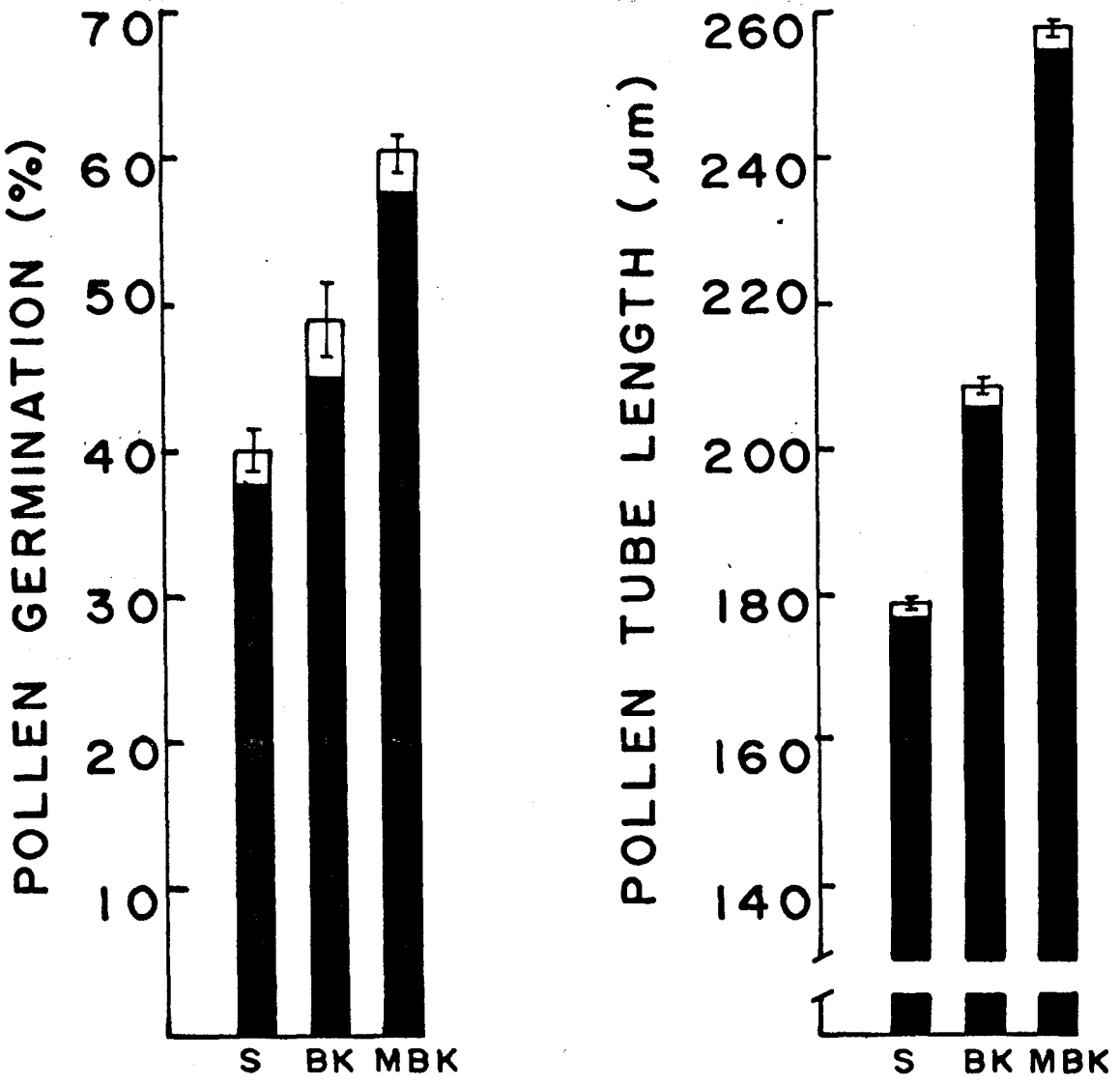

POLLEN GERMINATION (%)
80
70
60
50
40
30
20
10
0
POLLEN TUBE LENGTH (μm)
320
280
240
200
160
120
80
40
0
0 0·2 0·6 1·0 1·4 1·8
0 0·2 0·6 1·0 1·4 1·8
AGAR CONCENTRATION (% W/V)

Fig.16

Fig. 17 : Effect of different pollen germination media on S. marginatum pollen germination and pollen tube elongation.

I SE

S=SUCROSE 15%
BK=BREWBAKER & KWACK'S (1963) MEDIUM
MBK=MODIFIED BREWBAKER & KWACK MEDIUM



MEDIUM

Fig.17

Fig. 18a : Effect of growth hormones (Kinetin - **KN**, indoleacetic acid - **IAA** and gibberellic acid - GA_3) on S. khasianum pollen germination.

I SE

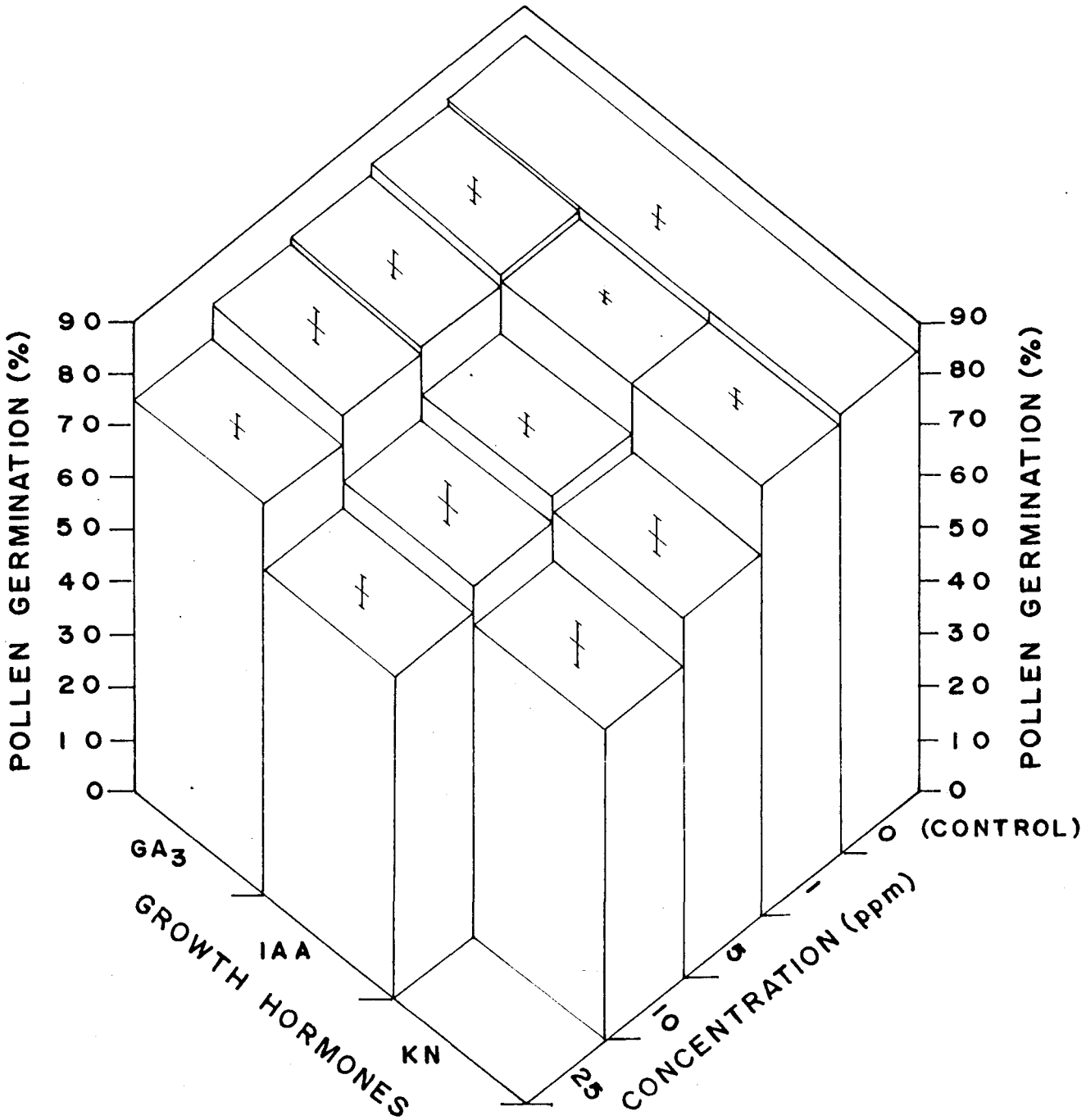

POLLEN GERMINATION (%)
0
10
20
30
40
50
60
70
80
90
GA3
IAA
KN
GROWTH HORMONES
25
10
5
1
0 (CONTROL)
CONCENTRATION (ppm)
POLLEN GERMINATION (%)

Fig.18a

Fig. 18b : Effect of growth hormones (Kinetin - KN, indoleacetic acid - IAA and gibberellic acid - GA_3) on S. khasianum pollen tube elongation.

I SE

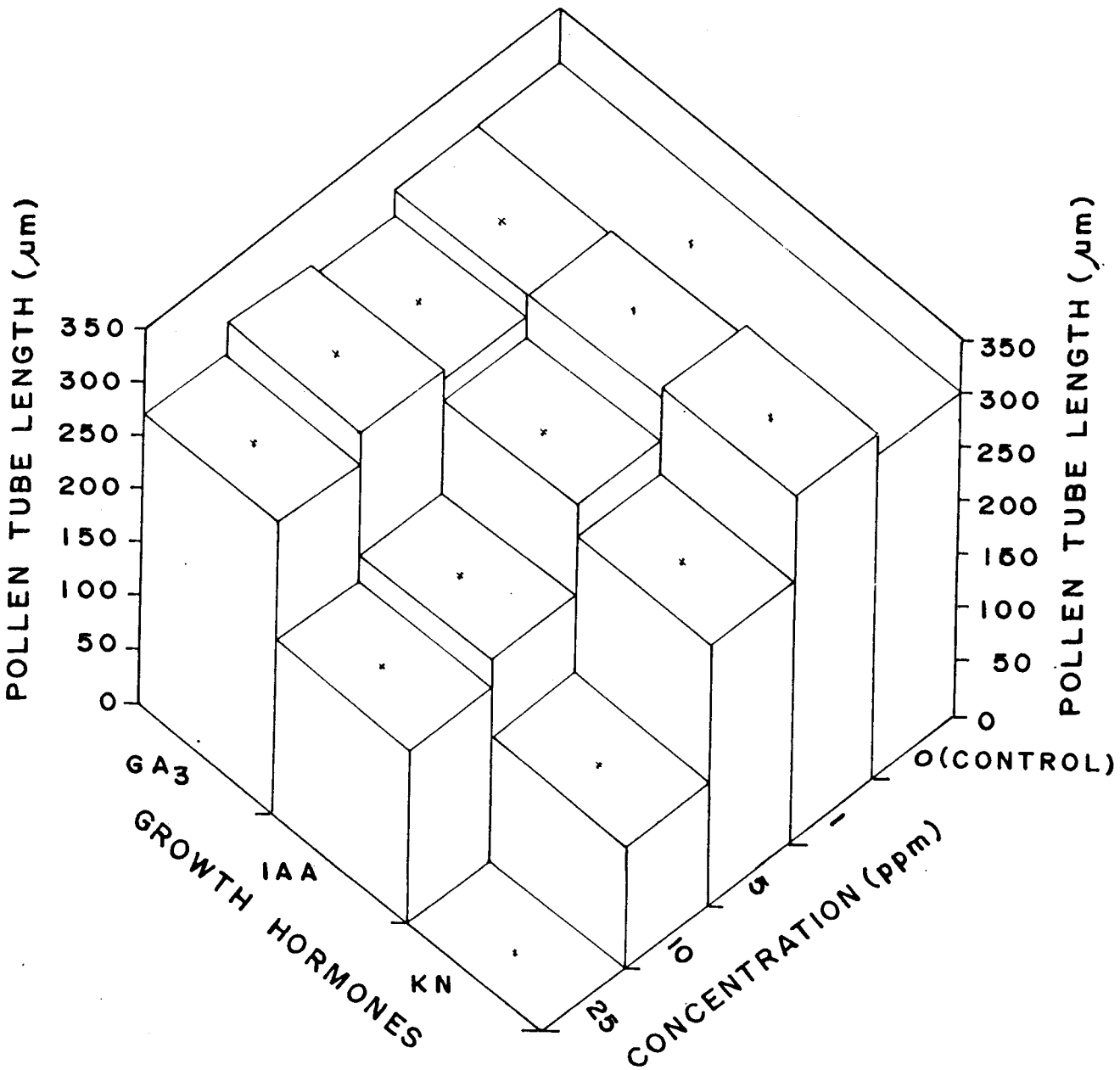

POLLEN TUBE LENGTH (µm)
350
300
250
200
150
100
50
0
GA3
IAA
KN
GROWTH HORMONES
25
10
5
1
0(CONTROL)
CONCENTRATION (ppm)
POLLEN TUBE LENGTH (µm)

Fig.18b

Fig. 19a : Effect of growth hormones (Kinetin - **KN**, indoleacetic acid - **IAA** and gibberellic acid - GA_3) on S. marginatum pollen germination.

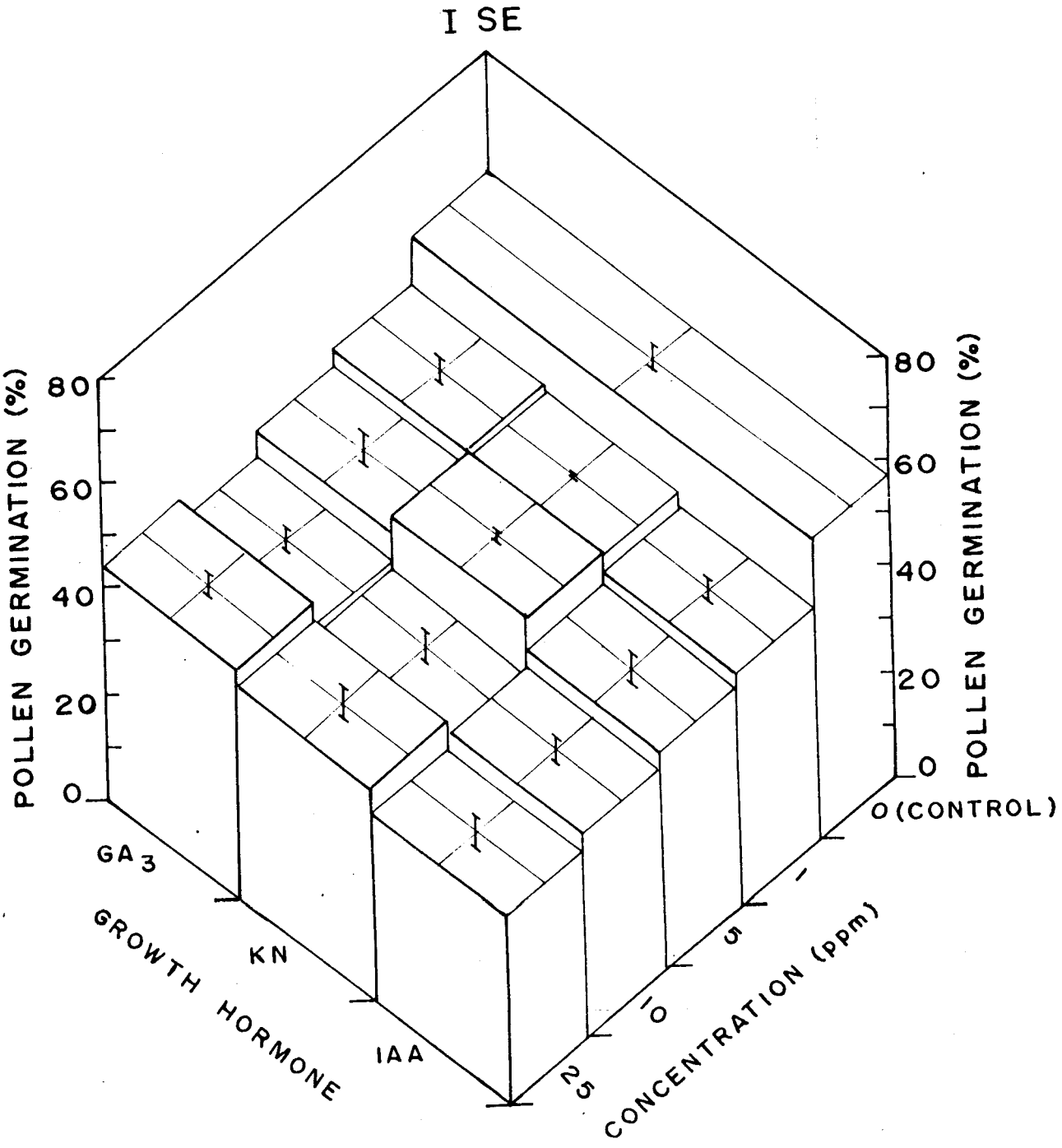

I SE
POLLEN GERMINATION (%)
80
60
40
20
0
GA3
KN
IAA
GROWTH HORMONE
2.5
10
5
-
CONCENTRATION (ppm)
0 (CONTROL)
POLLEN GERMINATION (%)

Fig. 19 a

Fig. 19b : Effect of growth hormones (Kinetin - KN, indoleacetic acid - IAA and gibberellic acid - GA_3) on S. marginatum pollen tube elongation.

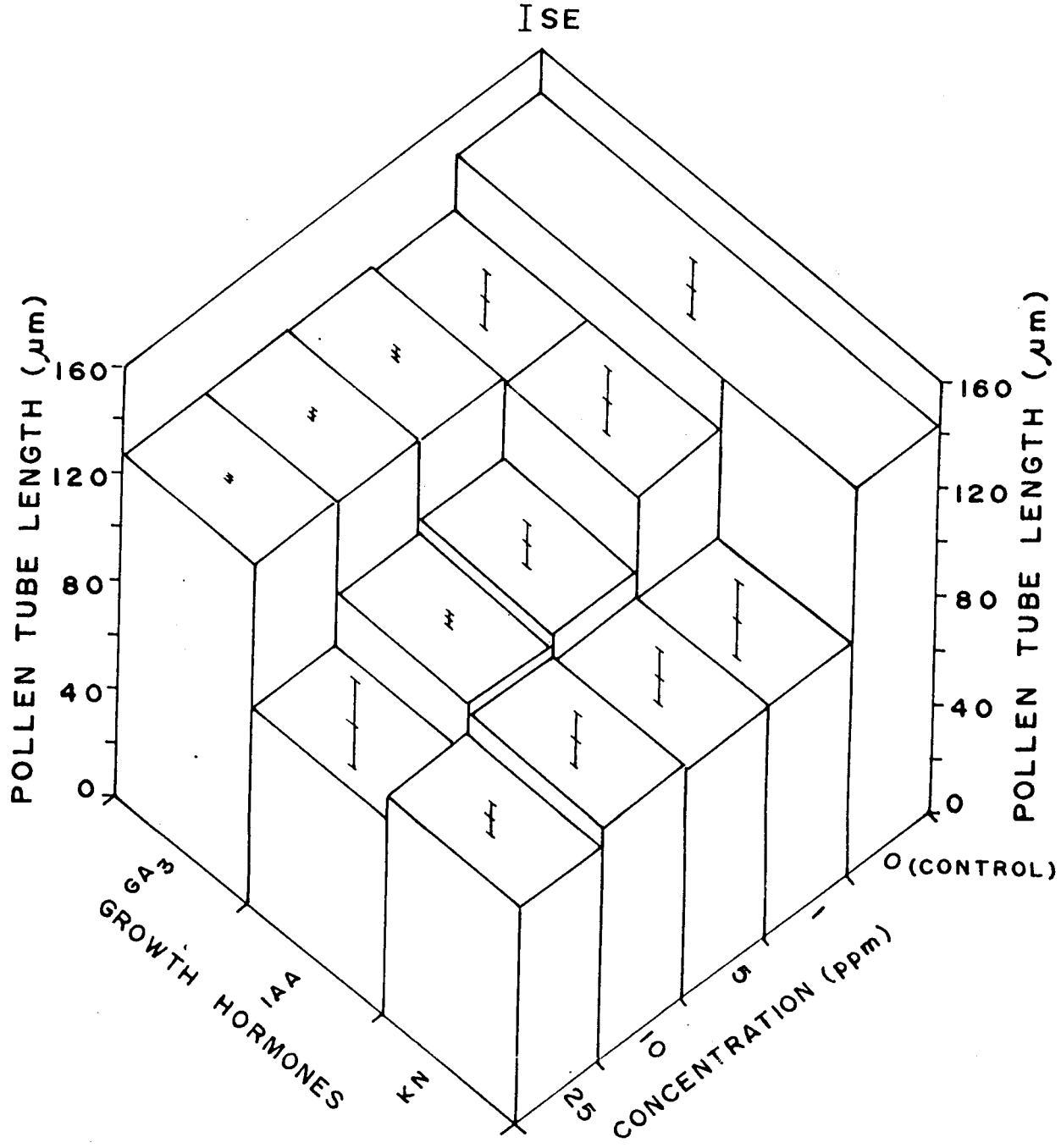

ISE
POLLEN TUBE LENGTH (μm)
160
120
80
40
0
GA3
IAA
KN
GROWTH HORMONES
25
10
5
1
0 (CONTROL)
CONCENTRATION (ppm)

Fig.19b

Fig. 20.1 : Effect of gamma radiation on S. khasianum pollen germination and pollen tube elongation.

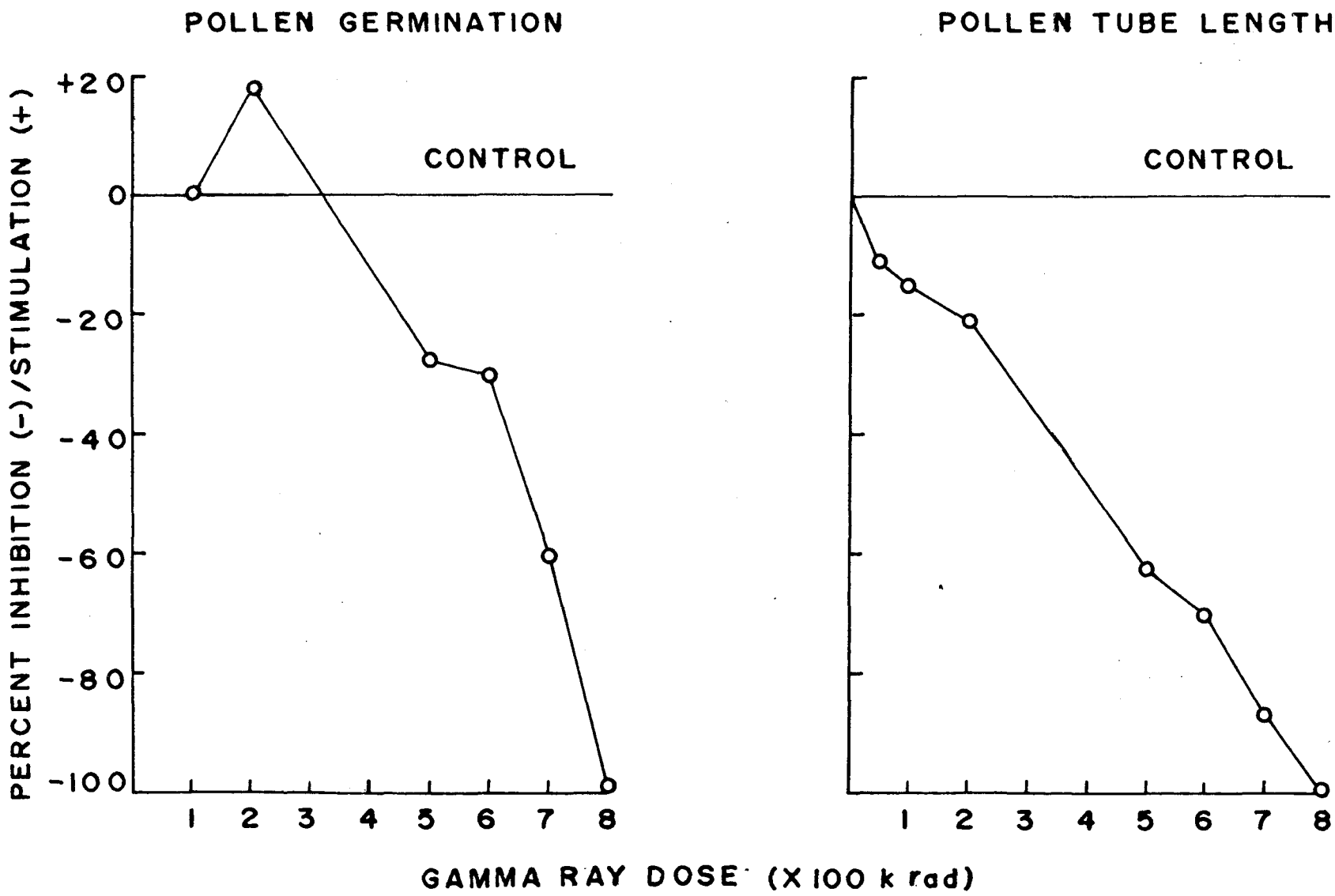

POLLEN GERMINATION
POLLEN TUBE LENGTH
PERCENT INHIBITION (-)/STIMULATION (+)
+20
0
-20
-40
-60
-80
-100
CONTROL
CONTROL
1 2 3 4 5 6 7 8
1 2 3 4 5 6 7 8
GAMMA RAY DOSE (X 100 k rad)

Fig. 20·1

Fig. 20.2 : Effect of gamma radiation on S. marginatum pollen germination and pollen tube elongation.

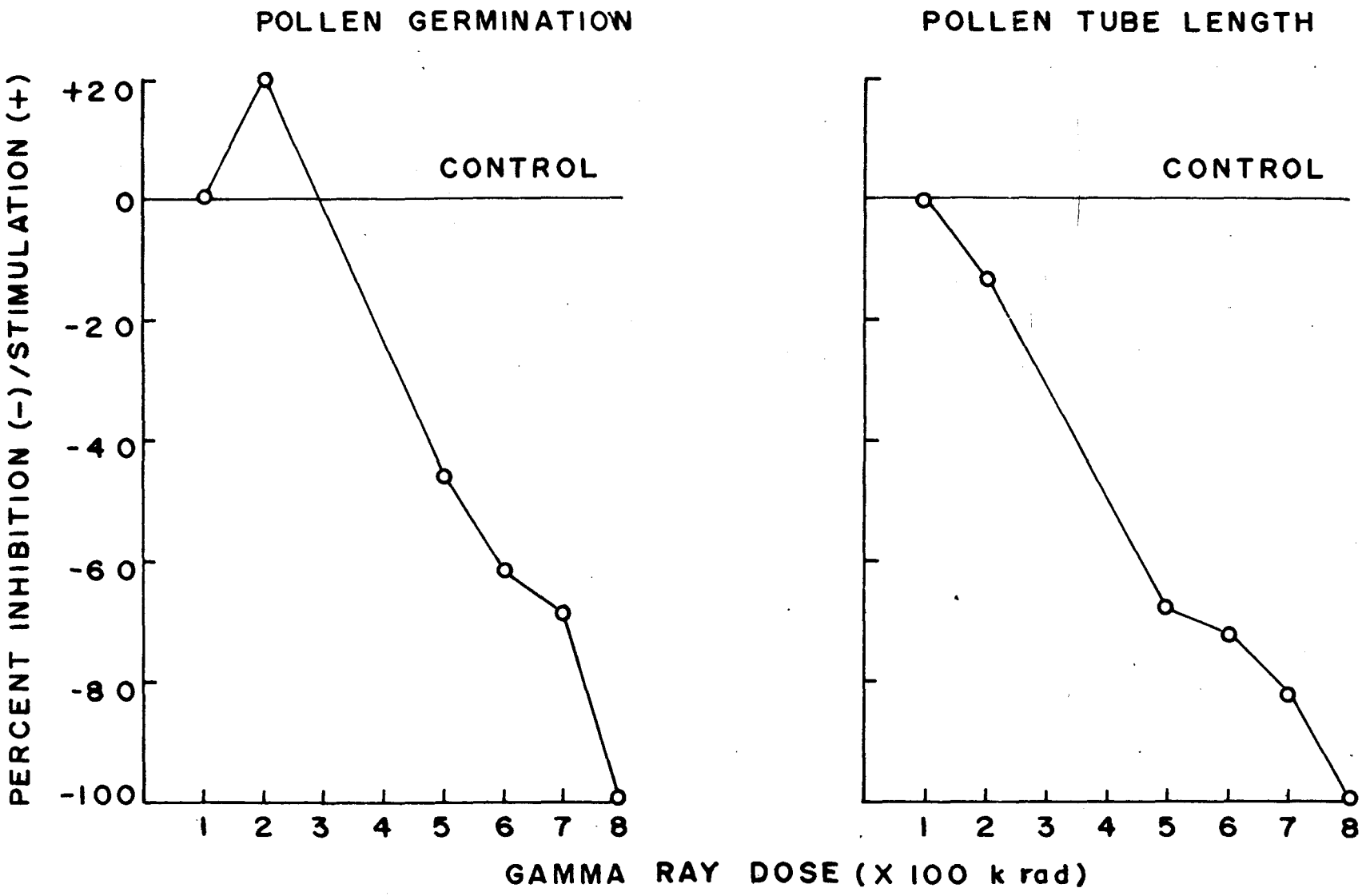

POLLEN GERMINATION
POLLEN TUBE LENGTH
PERCENT INHIBITION (-)/STIMULATION (+)
+20
0
-20
-40
-60
-80
-100
CONTROL
CONTROL
1 2 3 4 5 6 7 8
1 2 3 4 5 6 7 8
GAMMA RAY DOSE (X 100 k rad)

Fig.20-2

Fig. 21.1a : Modulation of radiation effect on pollen germination of S. khasianum, using growth hormones.

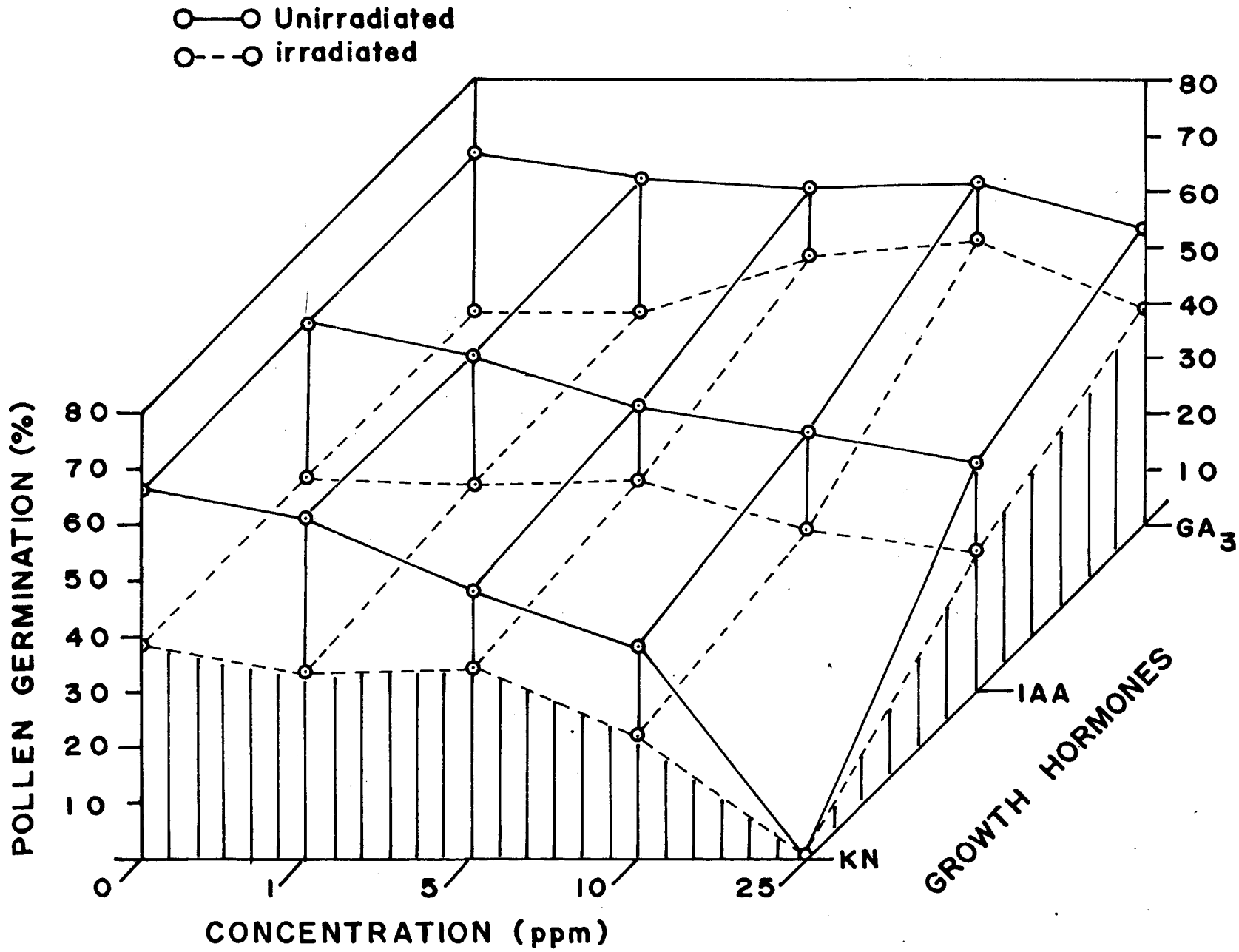

Unirradiated
irradiated
POLLEN GERMINATION (%)
80
70
60
50
40
30
20
10
0
1
5
10
25
CONCENTRATION (ppm)
KN
IAA
GA3
GROWTH HORMONES

Fig. 21·1a

Fig. 21.1b : Modulation of radiation effect on pollen tube elongation of S. khasianum, using growth hormones.

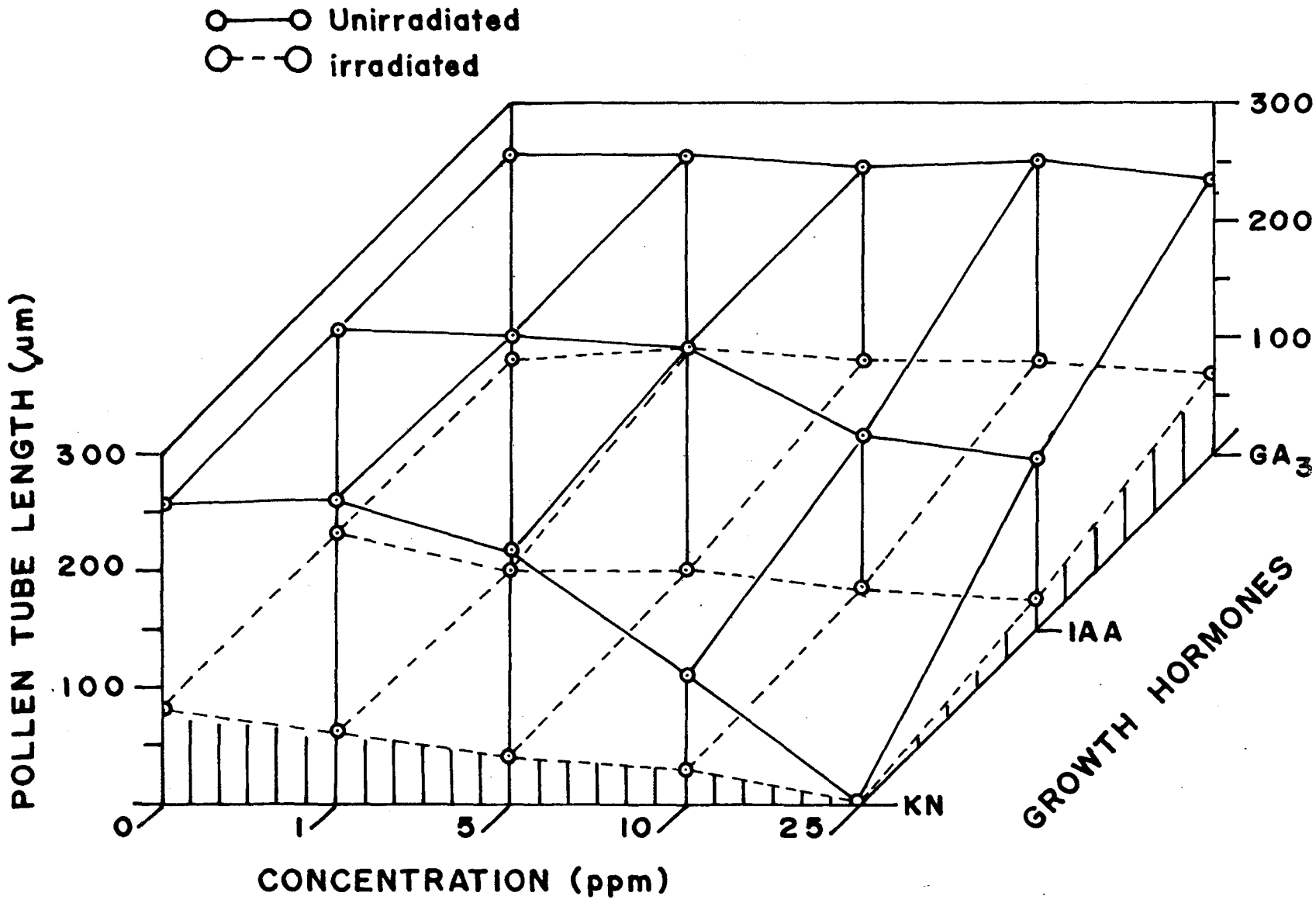

Unirradiated
irradiated
POLLEN TUBE LENGTH (µm)
300
200
100
0
1
5
10
25
CONCENTRATION (ppm)
KN
IAA
GA3
GROWTH HORMONES

Fig.21·1b

Fig. 21.2a : Modulation of radiation effect on pollen germination of S. marginatum pollen germination, using growth hormones.

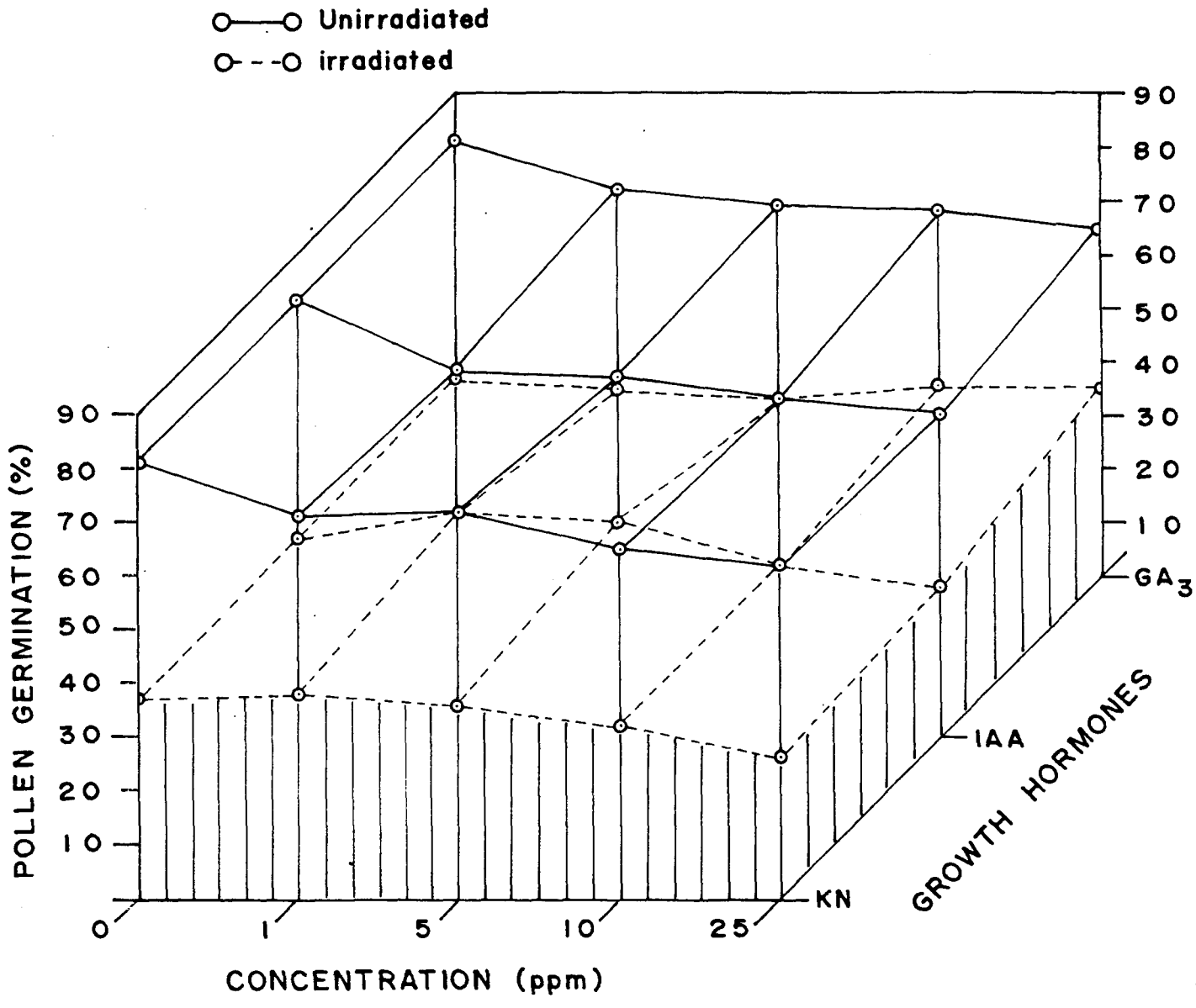

Unirradiated
irradiated
POLLEN GERMINATION (%)
90
80
70
60
50
40
30
20
10
0
1
5
10
25
CONCENTRATION (ppm)
KN
IAA
GA3
GROWTH HORMONES

Fig. 21·2a

Fig. 21.2b : Modulation of radiation effect on pollen tube elongation of S. marginatum, using growth hormones.

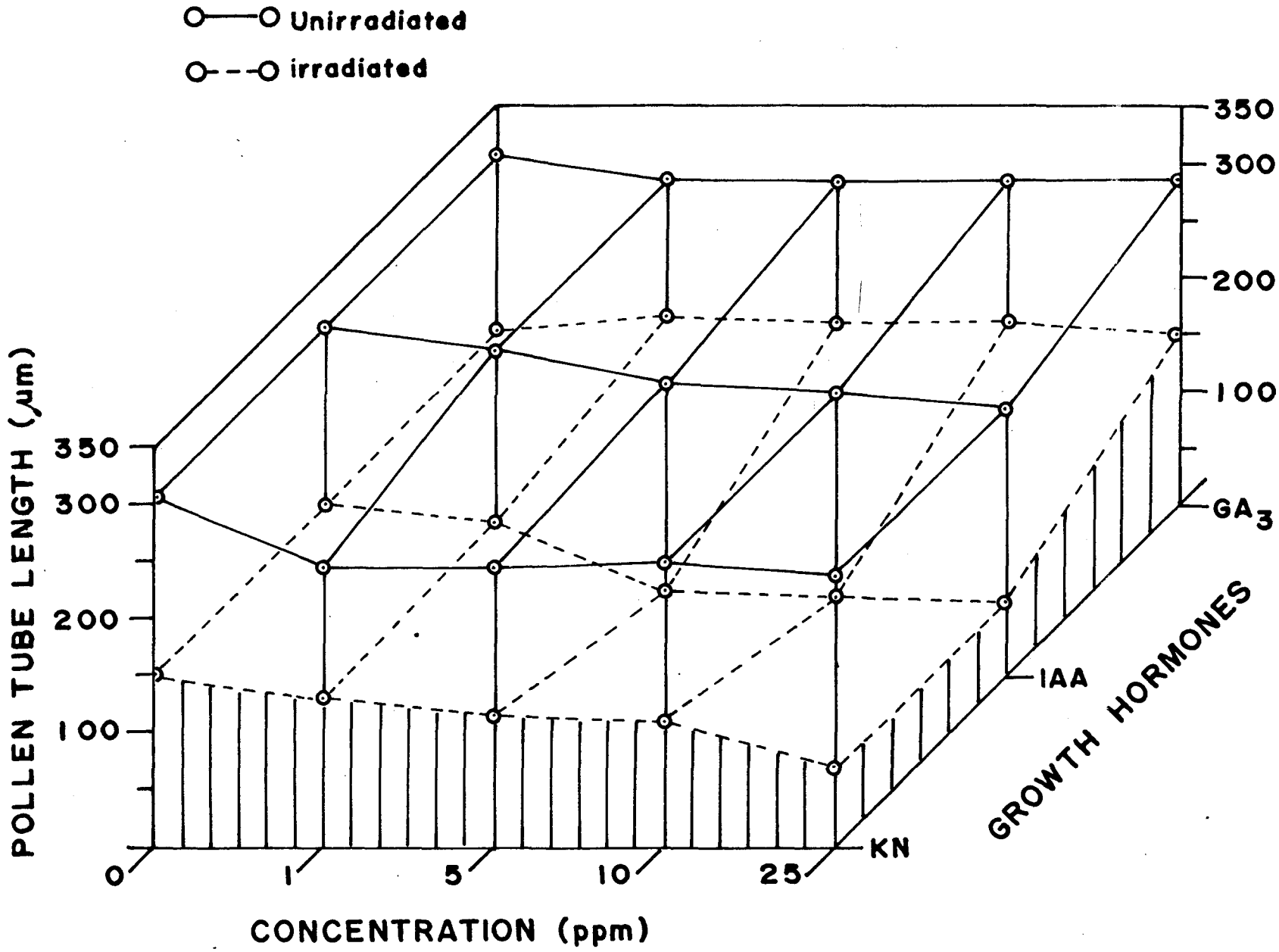

Unirradiated
irradiated
POLLEN TUBE LENGTH (µm)
350
300
200
100
0
1
5
10
25
CONCENTRATION (ppm)
KN
IAA
GA3
GROWTH HORMONES

Fig.21·2b

Fig. 22.1 : Effect of growth hormones (10 ppm), gamma radiation (600 krad) and their combinations on insoluble polysaccharides in 2 hr incubated pollen tubes of S. khasianum (All figures x 10,000).

a - Control
b - 10 ppm Kinetin
c - 10 ppm IAA
d - 10 ppm GA_3
e - 600 krad
f - 600 krad + 10 ppm Kinetin
g - 600 krad + 10 ppm IAA
h - 600 krad + 10 ppm GA_3.

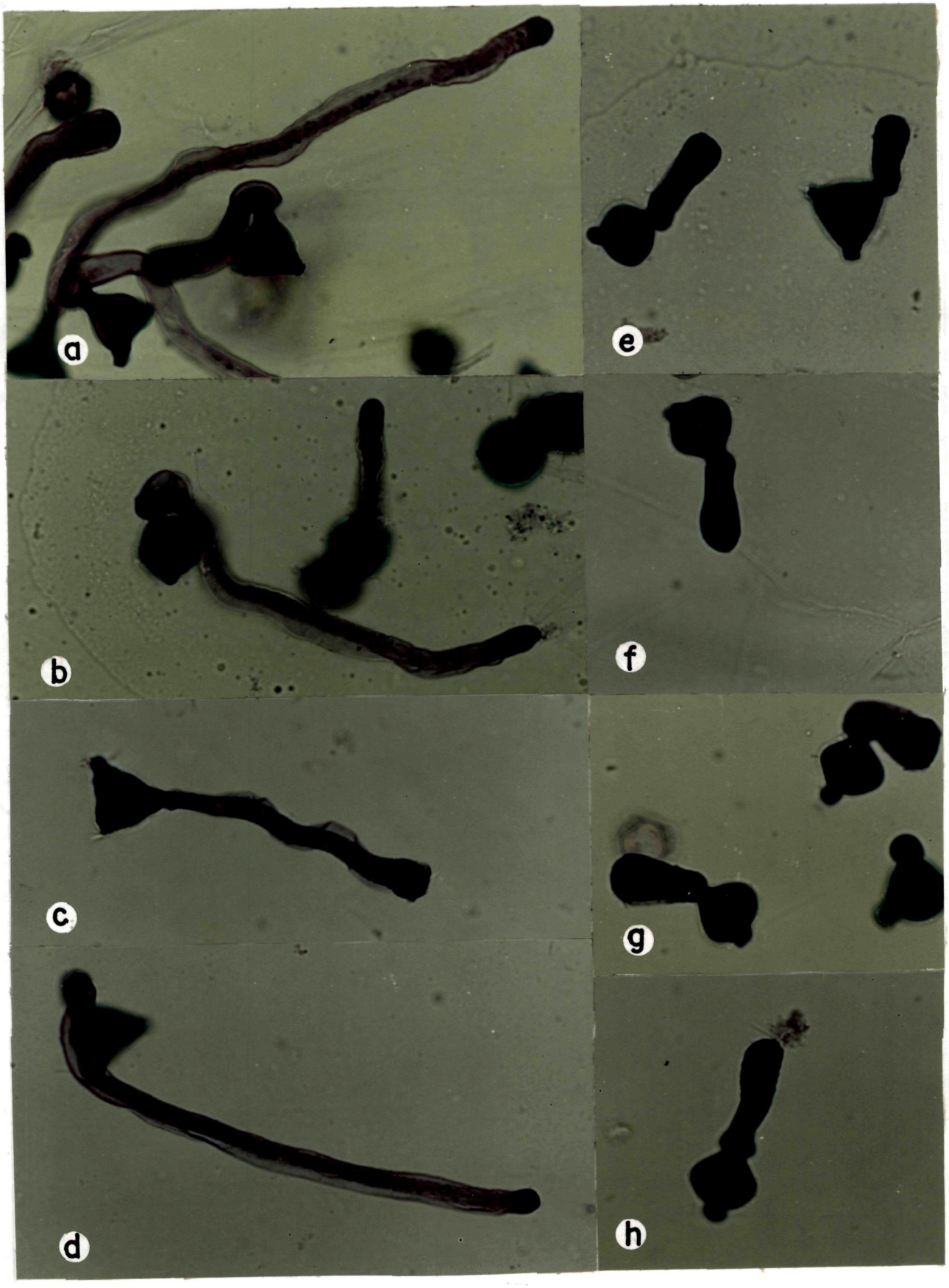


Fig. 22·1

Fig. 22.2 : Effect of growth hormones (10 ppm), gamma radiation (600 krad) and their combinations on protein in 2 hr incubated pollen tubes of S. khasianum (All figures **x 10,000**).

a - Control
b - 10 ppm Kinetin
c - 10 ppm IAA
d - 10 ppm GA_3
e - 600 krad
f - 600 krad + 10 ppm Kinetin
g - 600 krad + 10 ppm IAA
h - 600 krad + 10 ppm GA_3.

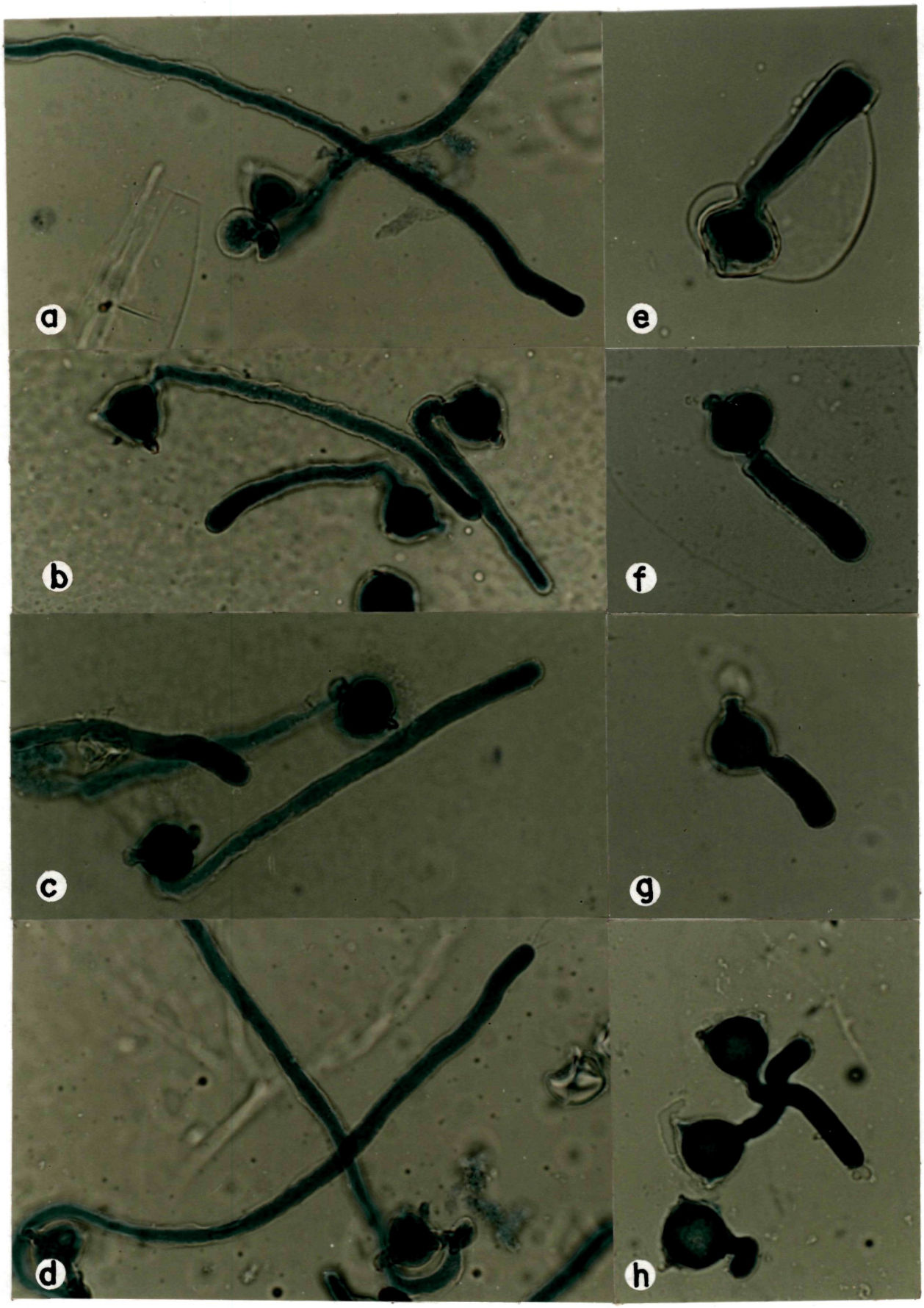


Fig. 22·2

Fig. 22.3 : Effect of growth hormones (10 ppm), gamma radiation (600 krad) and their combinations on nucleic acid (RNA) in 2 hr incubated pollen tubes of S. khasianum (All figures x 10,000).

a - Control
b - 10 ppm Kinetin
c - 10 ppm IAA
d - 10 ppm GA_3
e - 600 krad
f - 600 krad + 10 ppm Kinetin
g - 600 krad + 10 ppm IAA
h - 600 krad + 10 ppm GA_3.

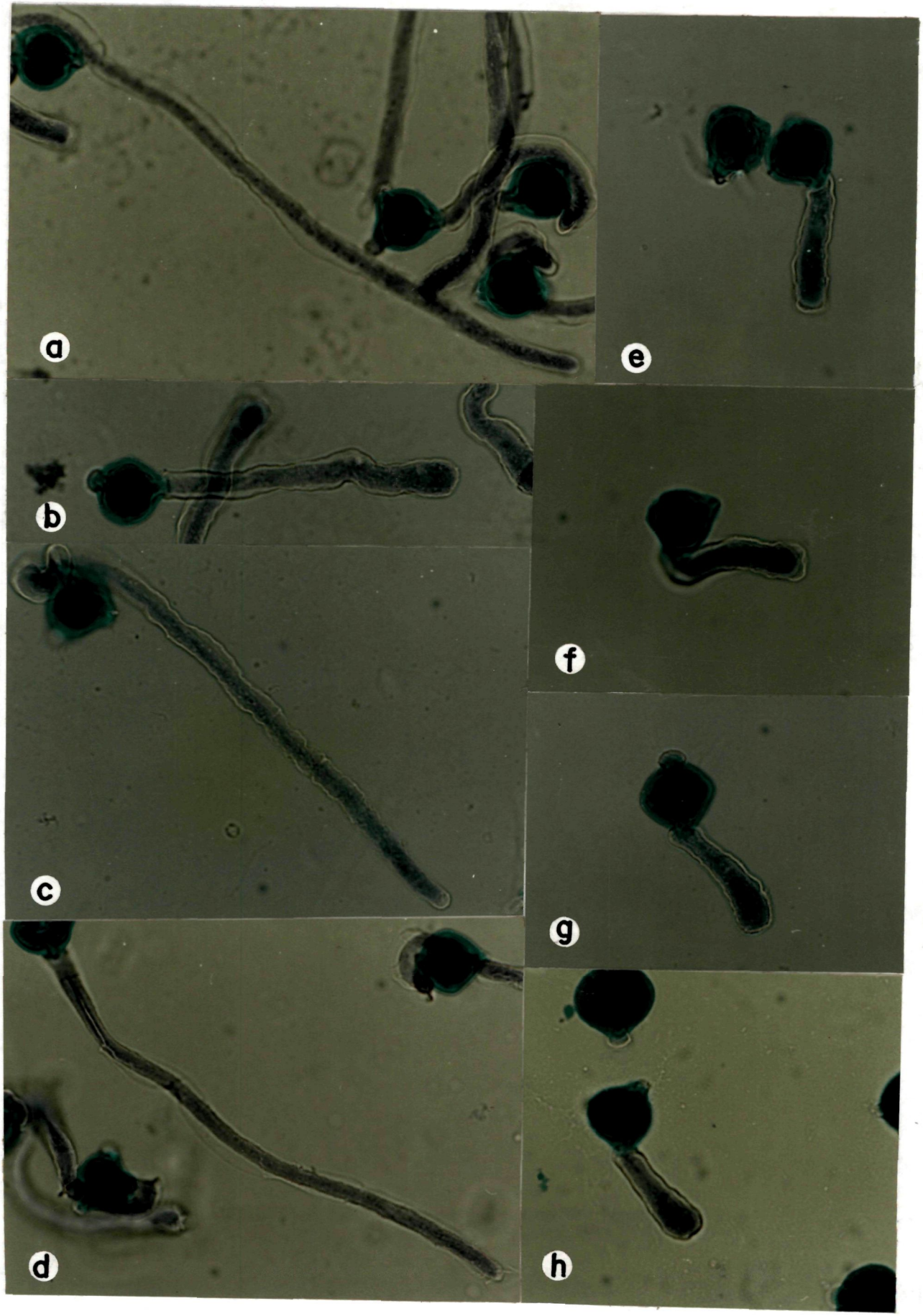


Fig. 22·3

Fig. 23.1 : Effect of growth hormones (10 ppm), gamma radiation (500 krad) and their combinations on insoluble polysaccharides in 2 hr incubated pollen tubes of S. marginatum (All figures **x 10,000**).

a - Control
b - 10 ppm Kinetin
c - 10 ppm IAA
d - 10 ppm GA_3
e - 500 krad
f - 500 krad + 10 ppm Kinetin
g - 500 krad + 10 ppm IAA
h - 500 krad + 10 ppm GA_3.

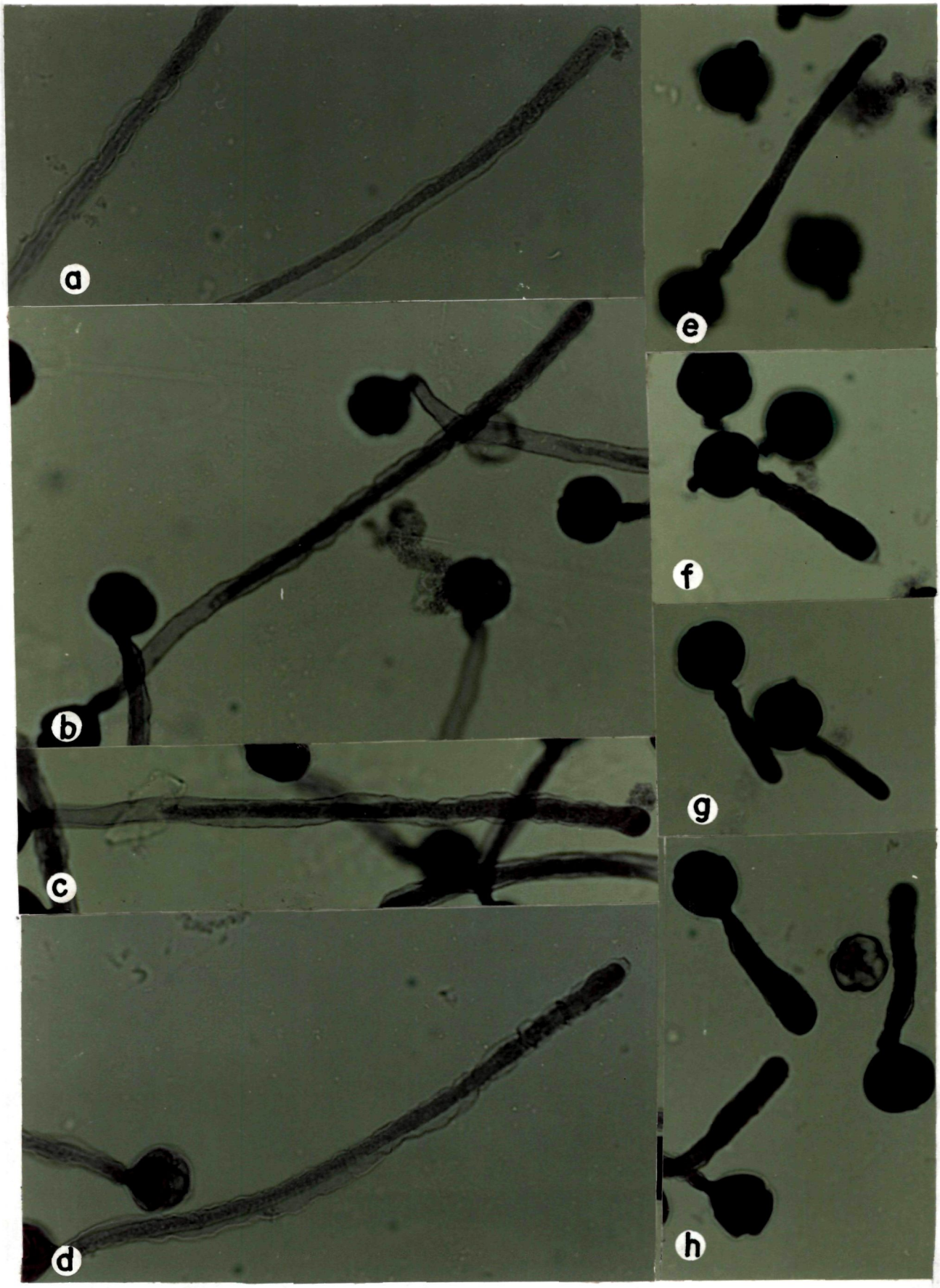


Fig. 23·1

Fig. 23.2 : Effect of growth hormones (10 ppm), gamma radiation (500 krad) and their combinations on protein in 2 hr incubated pollen tubes of S. marginatum (All figures x 10,000).

a - Control
b - 10 ppm Kinetin
c - 10 ppm IAA
d - 10 ppm GA_3
e - 500 krad
f - 500 krad + 10 ppm Kinetin
g - 500 krad + 10 ppm IAA
h - 500 krad + 10 ppm GA_3.

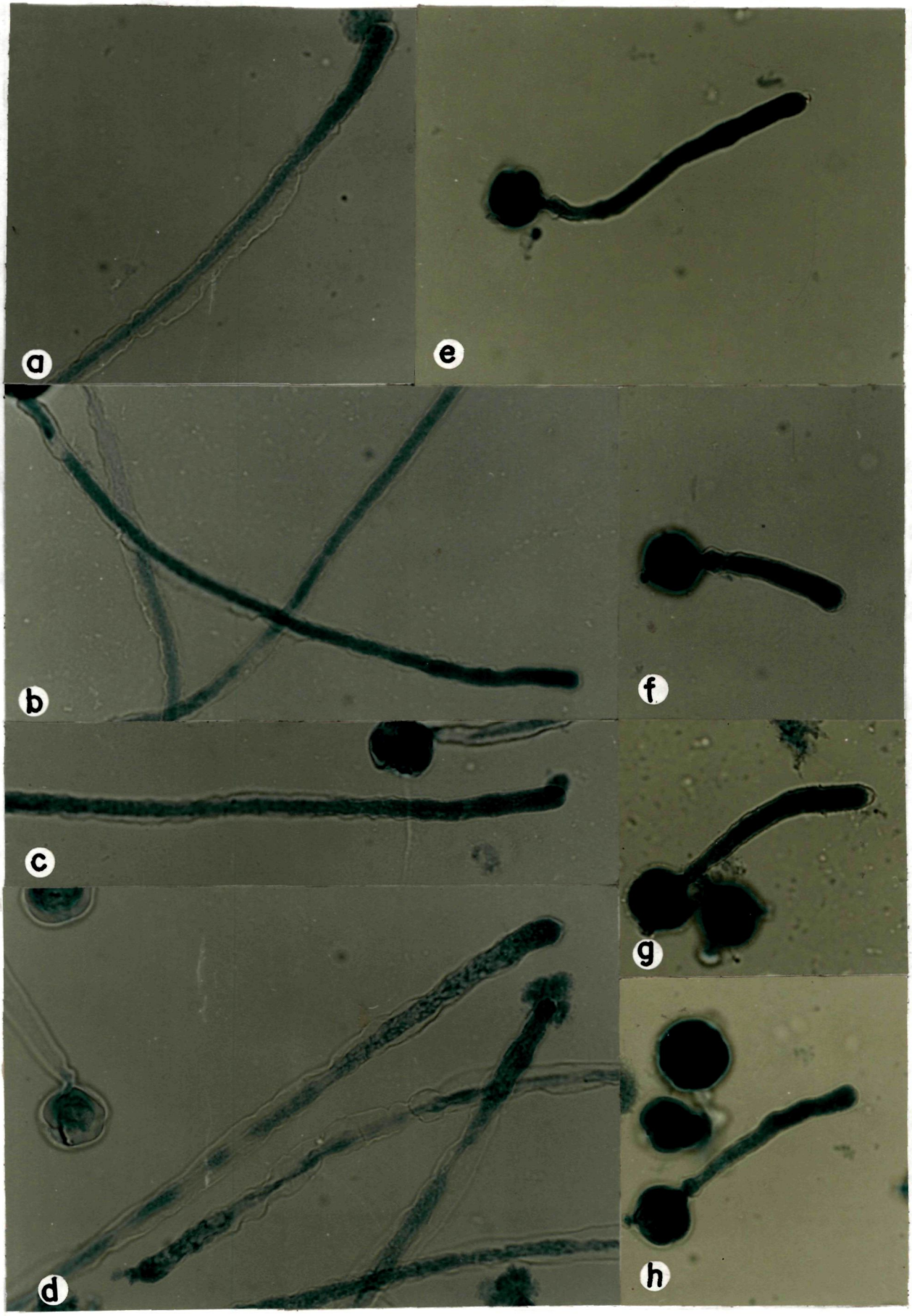


Fig. 23·2

Fig. 23.3 : Effect of growth hormones (10 ppm), gamma radiation (500 krad) and their combinations on nucleic acid (RNA) in 2 hr incubated pollen tubes of S. marginatum (All figures **x 10,000**).

a - Control
b - 10 ppm Kinetin
c - 10 ppm IAA
d - 10 ppm GA_3
e - 500 krad
f - 500 krad + 10 ppm Kinetin
g - 500 krad + 10 ppm IAA
h - 500 krad + 10 ppm GA_3.

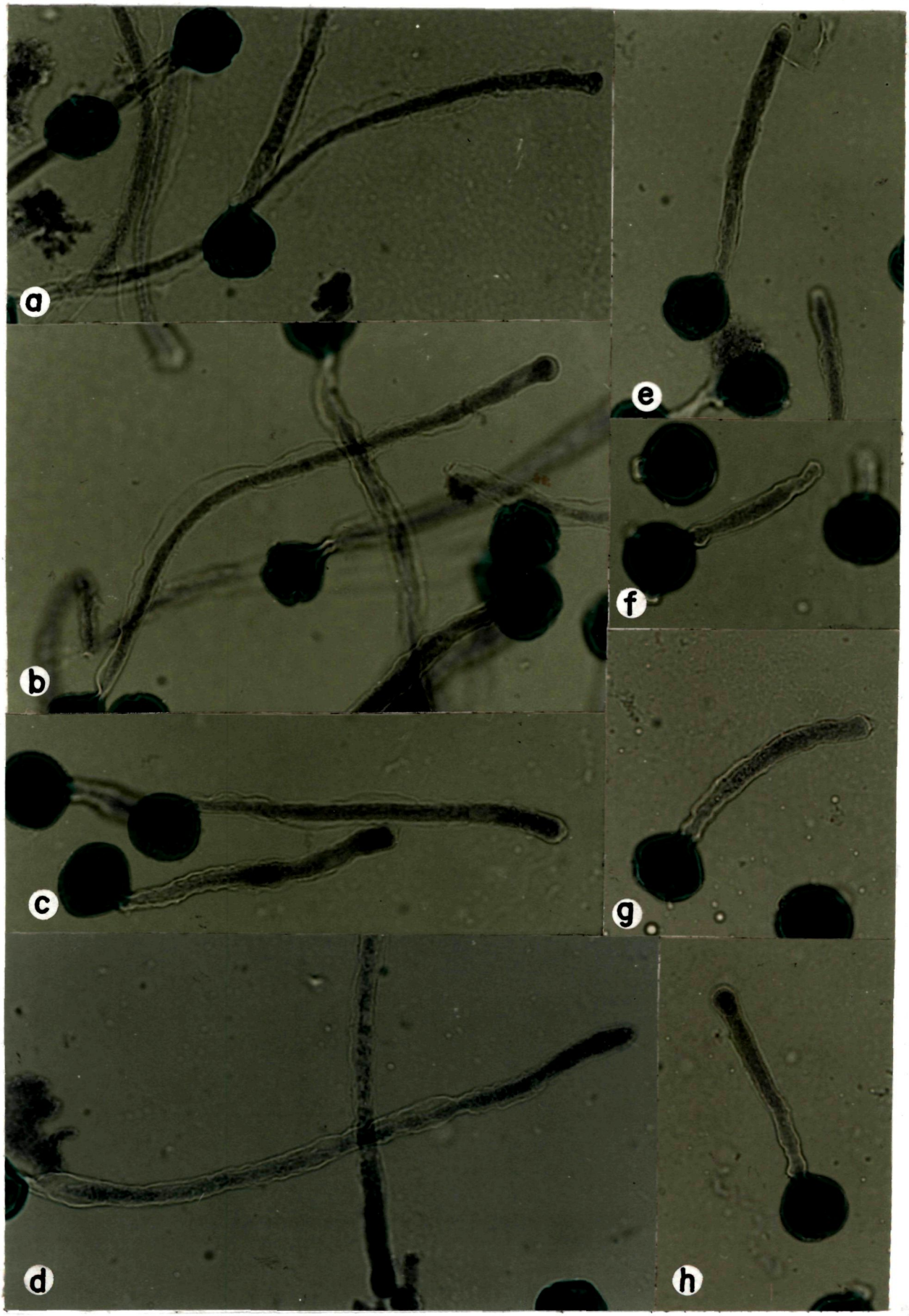


Fig. 23·3

Chapter-IV

FERTILIZING ABILITY OF IRRADIATED POLLEN

INTRODUCTION

Pollen irradiation has been used successfully to induce cytological and morphological variations in many herbaceous plants and the works have been critically reviewed by Brewbaker and Emery (1962).

Artificial cross-pollination using irradiated pollen has been carried out primarily for mutation breeding (Devreux et al., 1972), interspecific crosses (Stettler, 1968), haploid plant production (Lacadena, 1974) and induction of parthenogenesis (Cao et al., 1979). However, pollen irradiation affects the production of F_1 (first filial) embryos and viable F_1 seeds primarily causing cytogenetic damage and/or dominant lethals (Catcheside, 1948). Brewbaker and Emery (1962) stated that pollen irradiation lead to the impairment of viable

seed production primarily via its effects on (a) pollen germination, (b) sperm formation and pollen tube penetration of the ovules, (c) zygote formation and embryo growth, (d) endosperm development, (e) viability of mature seed and (f) delayed death in seedlings. Extensive damage to the embryo and seed in date palm as a result of pollen irradiation with high doses of gamma rays have been reported by Kgazal (1989).

It has been demonstrated by many investigators that pollen exposed to low level of ionizing radiation do not impair pollen tube in participating in fertilization and forming fruits (Brown and Cave, 1954; Pfahler, 1967; Vassileva-Dryanovska, 1966). Vassileva-Dryanovska (1966) observed that pollen exposed to 500 krad was able to penetrate the embryo sac. Some studies had indicated the formation of parthenocarpic fruits as a result of pollen exposures to ionizing radiations (Rick, 1943; Nishiyama and Tsukuda, 1959; Kgazal, 1989). Irradiated pollen although, do not affect fruit formation it apparently reduces seed set and seed viability in proportion to exposure (Uematsu and Nishiyama, 1967; Constant et al., 1971; Clausen, 1973a). However, fertilizing ability of irradiated pollen and subsequent effects on fruit formation and seed set have not been investigated so far in S. khasianum and S. marginatum.

Therefore, in the present research work the following aspects were considered :

1. Fertilizing ability of pollen irradiated with various doses of gamma rays in S. khasianum and S. marginatum.

2. Fruit setting and morphological characters of berries formed as a result of pollen irradiation in both the species.

3. Effect of pollen irradiation on seed setting.

MATERIALS AND METHODS

For determining the fertilizing ability and fruit forming capability of pollen irradiated with various doses of gamma radiation, anthesized flowers of both S. khasianum and S. marginatum were subjected to 5, 25, 50, 100, 200, 350, 500 and 700 krad doses of gamma rays. Long-styled flowers of both the species were emasculated just before anthesis and bagged with polyethylene bags. For aeration the bags were pierced with needle. Unirradiated (control) and irradiated pollen grains were kept in clean petriplate and at anthesis the stigma were dipped into these pollen grains. The pollinated flowers were protected against foreign pollen till the corolla dropped down. At least 100 flowers were pollinated for each treatment. The pollinated flowers were allowed to

form fruits and mature on the mother plant. Ripen fruits were harvested to assess fruit and seed development.

Few pollinated flowers from each treatment were also collected at different time intervals to assess pollen tube entry and growth in stigma, style and ovules. Some flowers were also used to evaluate post-fertilization changes in the ovules by sectioning the ovary.

Pollen Tube Entry

Pollinated flowers were collected at 48,72, and 96 hours time intervals and fixed in Carnoy's fluid (Abs. alcohol:Chloroform:glacial acetic acid - 6:4:1) for 24 hours. These flowers were then brought to water through descending alcohol series. For staining and clearing the fixed flowers, the method formulated by Alexander (1987) was adopted. The hydrated flowers were stained in staining mixture comprising 78 ml lactic acid, 4 ml malachite green (1%), 6 ml acid fuchsin (1%), 4 ml aniline blue (1%), 2 ml Orange G (1%) and 5 g chloral hydrate. The flowers were kept in the staining mixture for 24 hours at 45°C and then transferred to softening and clearing solution for another 24 hours. Softening and clearing solution consisted of 10 g phenol, 10 g chloral hydrate, 78 ml lactic acid and 2 ml Orange G (1%). The flowers were changed to fresh softening and clearing medium and hydrolyzed for 30 minutes at 58°C. The cleared and

softened flowers were washed two-three times in lactic acid and stored in lactic acid for later examination. The ovules were dissected out carefully and spread on a glass slide and mounted in a mixture of lactic acid and glycerol (1:1) for observation. Photomicrographs were taken with the help of Leitz Ortholux II microscope fitted with vario-orthomat photomicrography attachment.

Seed Development

Flowers pollinated with control and irradiated pollen were collected at 2, 3, 4, 5, 7 and 15 days interval and fixed in ethanol: acetic acid (3:1) for 24 hours to investigate seed development. These were dehydrated in Tertiary butanol series and infiltrated and embedded in paraffin. Sections 10-15 µm thick were cut using Leitz 1512 rotary microtome. Sections were deparaffinised and stained with Safranin-Aniline blue (Jensen, 1962). The sections were first stained in 0.5% Safranin (1% Safranin dissolved in 95% alcohol and diluted with an equal amount of distilled water just before use) for 24 hours. The slides were then washed in water and passed briefly through acidified 70% alcohol to remove excess stain, and then passed rapidly through 95% and absolute alcohol. The sections were counterstained with aniline blue (1% aniline blue in absolute alcohol, diluted 50% with clove oil before use) for 1 minute. The sections were then given two changes in a mixture of clove

oil, absolute alcohol and xylene (50:25:25) for 2-3 minutes. Finally, the slides were brought to pure xylene, changed twice and mounted in D.P.X.

Nucleoli, cuticle and lignified cell walls stained red while the remaining structures including cytoplasm stained blue. Photomicrographs were taken with the help of Leitz Ortholux II microscope fitted with vario-orthomat photomicrography attachment.

Fruit and Seed Formation

Percentage of fruit formation in flowers hand pollinated with unirradiated and irradiated (5-700 krad) pollen were assessed after one month of pollination. Ripe fruits were harvested for determining berry volume, fresh and dry weight of berry, number of seeds per berry and weight of 100 seeds. Berry volume was determined using slide caliper and the mean value was calculated following the formula $1/6\pi d^3$ (or $4/3\pi r^3$) where 'd' is the diameter (r=radius) of the berry. Two measurements from each berry were taken at two planes perpendicular to one another. For determining fresh weight these berries were weighed and then dried in a hot air oven (45°C) to find out dry weight. Moisture content of the berries was also calculated using the formula :

$$\frac{\text{Fresh wt. - Dry wt.}}{\text{Fresh wt.}} \times 100$$

Number of seeds per berry was also determined in each treatment. Percentage of filled seeds was determined by soaking the seeds in water in a beaker. Filled seeds settled down at the bottom while empty/rudimentary seeds floated on the surface of water. Mean weight of 100 seeds was determined by weighing 10 sets of 100 seeds for each treatment. Radiation-induced stimulation/inhibition over control was calculated as before.

RESULTS

S. khasianum

Data on fertilizing and fruit forming ability of control and irradiated (5-700 krad) pollen of S. khasianum are given in **Table 25.** Control and pollen irradiated with 5 krad of gamma radiation did not differ in their ability to induce fruit set. But higher doses of radiation (25-700 krad) adversely affected fruit forming ability of the pollen. Percentage of fruit formation decreased with the increasing dose and no fruiting occurred at 700 krad **(Table 25).** In control flowers the petals took 4-6 days to fall after pollination **(Table 25).** No difference was evident in senescence of petals of flowers pollinated with control and irradiated pollen (5-700 krad) **(Table 25).** Berries formed by control and irradiated pollen ripened simultaneously (90-120 days) **(Table 25).** **Table** 25 also

gives data on the effect of irradiated pollen on berry characters and moisture content of the berries. Control berries measured 2.90 cm in diameter and had a volume of 12.79 cm^3. The berries formed by irradiated (5-500 krad) pollen were smaller in size compared to control **(Table 25, Figs. 26a & b).** The volume of the berry diminished with the increasing radiation dose. Pollen irradiation also influenced fresh weight and dry weight of the berries **(Table 25).** Reduction in weight increased with the increasing radiation dose. At harvest the fresh weight of control berries was 10.84 g and dry weight 2.45 g **(Table 25).** The berries formed from irradiated pollen weighed less than the control and the percent inhibition of fresh and dry weight of the berries increased with the increasing dose of radiation **(Table 25).** Berries formed due to irradiated (5-500 krad) pollen had more moisture (88.72-91.40%) than control berries (77.40%) **(Table 25).**

Irradiated pollen greatly impaired seed set in S. khasianum **(Table 26, Fig. 26b).** Seed development occurred only when pollen irradiated with 5 krad of gamma rays were used for pollination. However, seed set even at this dose was 18.61% less than control **(Table 26).** All the pollen given higher doses of gamma rays (25-500 krad) produced seedless berries **(Table 26).** Although 5 krad of radiation supported seed setting, percent of filled seeds was less compared to control **(Table 26).** Similarly, in comparison

to control seeds, the seeds formed by 5 krad irradiated pollen weighed less **(Table 26)**.

In the present study pollen tubes of control and pollen irradiated with 5-100 krad of gamma rays entered ovules after 48 hours of pollination while tubes of pollen grains give 350 krad dose reached ovules after 96 hours of pollination **(Figs. 24.1a-f & 24.2a-g)**. The pollen tubes given 500 krad gamma radiation dose, however, did not reach ovule even after 96 hours **(Fig. 24.2g)**.

Longitudinal section of the pollinated ovary at different time intervals **(Figs. 25.1a-h)** indicated that in control and 5-200 krad irradiated pollen, the pollen tubes entered the embryo sac near the egg within 48 hours of pollination **(Figs. 25.1a-f)**. In control, 48 hours after pollination one of the synergids was dark and remained so upto 72 hours of pollination **(Figs. 25.1a & 25.2a)**. One of the synergids appeared shrunk and endospermic cells started developing 96 hours after pollination **(Fig. 25.3a)**. One hundred and twenty hours after pollination the synergids degenerated and the zygote was surrounded by endospermic tissue **(Fig.25.4a)**. One week after pollination, in control, ovules although endosperm was quite developed the zygote remained single celled **(Fig. 25.5a)**. On 15th day of pollination the control ovules revealed well developed endospermic tissue and 5-celled

proembryo (Figs. 25.6a & b). Changes occurring in the ovules pollinated with 5-200 krad irradiated pollen resembled development in control till one week of pollination (Figs.25.1b-f-25.5b-f). However, in these ovules division of secondary nucleus and the development of endospermic tissue was delayed (Figs.25.1b-f-25.5b-f). Pollen irradiated with 5 krad caused degeneration of seed in some ovules 15 days after pollination (Fig.25.6c). Pollen irradiated with 25-50 krad induced proliferation of the endothelial cells one week after pollination (Figs.25.5c & d). The endosperm development in ovules pollinated with 25-200 krad irradiated pollen was also hampered at the this stage (Figs.25.5c-f). The enlarged endothelial cells 15 days after pollination occupied whole embryo sac region in the ovules pollinated with 25-200 krad irradiated pollen (Figs.25.6d-g). Pollen irradiated with higher doses (350 and 500 krad) of gamma rays failed to induce fertilization, as a result the egg cell enlarged and the ovules collapsed one week after pollination (Figs.25.1g & h-25.5g & h).

S. marginatum

The data on fertilizing ability and fruit set caused by control and irradiated pollen (5-700 krad) of S. marginatum are given in Table 27. Control pollen set fruit in 66.67% pollinated flowers while only 26.32% fruits formed when flowers were pollinated with 5 krad

irradiated pollen (**Table 27**). Pollen irradiated with higher doses (25-700 krad) failed to induce fruiting in S. marginatum. No difference was evident in the senescence of petals in flowers pollinated with control and irradiated pollen (**Table 27**). Similarly, the ripening of berries set by irradiated pollen was not different from control (**Table 27**). Size, volume, fresh weight and dry weight of berries set due to pollination with 5 krad irradiated pollen also did not differ from control berries (**Table 27, Figs.29.1 & 2**). Berries formed due to 5 krad irradiated pollen, however, had higher moisture content than control berries (**Table 27**).

Table 28 gives data on seed setting in control and berries developed due to 5 krad irradiated pollen of S. marginatum. Seed setting was greatly impaired as a result of pollen irradiation and number of seeds per berry was less than control in berries formed due to 5 krad irradiated pollen (**Table 28**). Similarly, number of filled seeds per berry and weight of seeds in berries formed due to irradiated pollen were also significantly reduced (**Table 28**).

Pollen of S. marginatum irradiated with 5-100 krad of gamma radiation did not impair pollen tubes entry into the ovules (**Figs.27a-f**). Pollen tubes of both control and irradiated (5-100 krad) pollen entered the ovule within 48 hours of pollination (**Figs.27a-e**). However, pollen tube

entry was not evident in ovules pollinated with 350 krad irradiated pollen at 48 hours **(Fig. 27f)**.

Longitudinal sections of the ovules at different time intervals after pollination revealed that in ovules pollinated with control and 25-50 krad irradiated pollen, the tubes enter the ovules in 48 hours of pollination **(Figs.28.1a-c)** and 96 hours after pollination endospermic tissue started developing in control and ovules pollinated with 5 krad irradiated pollen **(Figs. 28.1d-f)**. In control ovules endospermic tissue started developing in 120 hours and became prominent in one week time but the egg/zygote remained single celled and the two synergids became less distinct **(Figs. 28.2a & d)**. However, in ovules pollinated with irradiated pollen (25-50 krad) the egg/zygote appeared disorganised and disintegrating. The secondary nuclei in ovules pollinated with 25-50 krad irradiated pollen appeared shrunk and degenerating after 120 hours of pollination **(Figs. 28.2b & c)**. The ovules pollinated with irradiated pollen (25-50 krad) became disorganised and collapsed within one week of pollination **(Figs. 28.2e & f)**.

DISCUSSION

Gamma irradiated pollen did not affect post pollination petal senescence in S. khasianum and S. marginatum. In S. khasianum petals took 4-6 days to

wither after pollination while S. marginatum petals required more time (5-7 days).

Irradiated pollen of both the species exhibited greatly reduced fruit forming ability and the degree of inhibition increased with the increasing dose of gamma rays. In S. khasianum pollen irradiated with 5-500 krad could induce fruit setting but pollen irradiated with 700 krad failed to form any fruit. Unlike S. khasianum, S. marginatum pollen irradiated with 25-700 krad could not induce any fruiting. Percent fruit set in flowers pollinated with even low dose (5 krad) irradiated pollen was greatly reduced. Irradiated pollen induced decreased fruit setting is reported in various cultivars of pear (Sniezko and Visser, 1987) and date palm (Kgazal, 1989).

Berry maturity occurred simultaneously in control and berries formed due to irradiated pollen in both the species studied in the present investigation. Early or delayed maturation of berry in S. khasianum and S. indicum as a result of seed irradiated with gamma rays was recorded by Ravindran (1981). However, the present study indicates that maturation of berry formed due to irradiated pollen was not affected in S. khasianum and S. marginatum.

In S. khasianum diameter and volume of berry formed from pollination with irradiated pollen was greatly reduced. Berry diameter became smaller with the

increasing dose of gamma rays, but berry volume remained almost constant in the berries formed from pollen irradiated with 200-500 krad. In S. marginatum berries formed due to pollination with 5 krad irradiated pollen had smaller diameter and lesser volume compared to control berries but the values were not significantly different from control. The seeds developing in a fruit have a bearing on its development and growth; fruits having less seeds exhibit reduced growth in comparison to fruits having more number of seeds (Nitsch, 1965). It is suggested that seeds regulate growth of fruits by producing major portion of growth regulators found in fruits such as IAA or precursors of IAA, gibberellins and cell division factors (Nitsch, 1965). Radiation-induced decrease in fruit size due to seed and pollen irradiation has been reported in S. khasianum and S. indicum (Ravindran, 1981) and S. torvum (Chauhan and Katiyar, 1990).

Both fresh and dry weight of berry formed by irradiated pollen significantly decreased in S. khasianum. In S. marginatum fresh and dry weight of berries formed by 5 krad irradiated pollen was although less than control, the difference was not significant. There is a linear relationship between developing seeds and weight of fruit (Nitsch, 1965). This may hold true in the present study as well. Radiation-induced reduction in berry weight in S. khasianum and S. indicum has also been reported

earlier (Chauhan et al., 1975; Chauhan, 1978; Ravindran, 1981).

In the present study irradiated pollen induced smaller berries, however, moisture content in these berries was much higher than control berries of S. khasianum and S. marginatum.

Number of seeds produced per berry in both the species was greatly reduced due to pollination with irradiated (5 krad) pollen. In S. khasianum, although berries were formed in flowers pollinated with 25-500 krad irradiated pollen, all the berries were seedless. Reduced seed set due to pollination with irradiated pollen has been reported in many plant species (Brewbaker and Emery, 1962; Sniezko and Visser, 1987; Chin and Gordon, 1979). At this stage it becomes apparent that gamma irradiated pollen induced inhibition of seed development affects the growth and development of berries in the two tested species. As such the weight of berries was also reduced in berries with fewer seeds or no seeds. Development of seedless fruits due to irradiated pollen is reported in pear (Sniezko and Visser, 1987) and date palm (Kgazal, 1989). Seedless fruits may arise due to (1) pollination stimulus without fertilization (Parthenocarpy) and (2) fusion of gametes taking place but the zygote/embryo degenerating in the early stage of development (Nitsch,

1965). In the present study both these phenomena appear to be operating. In both S. khasianum and S. marginatum pollen irradiated with 5 krad of gamma rays did not hamper fertilization. However, some of the embryo degenerated in the later stage of development. Hence, empty/chaffed seeds developed in the berry. In S. khasianum ovules pollinated with 25-200 krad irradiated pollen, changes occurring in the embryosac upto one week time of pollination were similar to control although there was delay in endosperm development. Interestingly, in ovules pollinated with 25-200 krad irradiated pollen the endothelial cells enlarged and proliferated. These proliferated cells probably digested the endosperm and caused death to the zygote/embryo during early stages of development. As a result no seed rudiments were visible in these berries. Such proliferating tumorous tissue of endothelium leading to the abortion of embryos had been reported in incompatible crosses of Datura stramonium x D. metal (Rappaport, 1965) and inter-specific crosses of Solanum (Lee and Cooper, 1958). Auxin (IAA) has been suggested to be responsible for such tumors (Rappaport, 1965; Klein, 1965). Early collapsing of endothelium and enlarged egg cell in ovules pollinated with 350-500 krad irradiated pollen in S. khasianum suggests that fertilization did not occur but pollination stimulus caused parthenocarpic berries. In S. marginatum in ovules

pollinated with 25 and 50 krad irradiated pollen, tubes entered ovule but the egg/zygote appeared disorganised and disintegrating. The secondary nucleus appeared shrunk and degenerating. The ovules as a whole became disorganised and collapsed within one week of pollination which could be the reason for non-development of fruits at these doses.

Table 25. Effect of gamma irradiated pollen on berry formation in S. khasianum

Treatment	Senescence of petal after pollination (days)	Fruit set (%)	Fruit ripening (days)	Berry diameter (cm)	Berry volume (cm^3)	Berry fresh weight (g)	Berry dry weight (g)	Moisture content (%)
Control	4-6	96	90-120	2.90±0.04	12.79±0.50	10.84±0.78	2.45±0.85	77.40±1.53
5 krad	4-6	94	90-120	2.56±0.11*** (-11.72)	9.21±1.06*** (-27.99)	7.56±1.10*** (-30.26)	0.65±0.05*** (-73.47)	91.40±1.13***
25 krad	4-6	71	90-120	2.45±0.08*** (-15.52)	7.96±0.83*** (-37.76)	5.25±0.70*** (-51.57)	0.52±0.10*** (-78.78)	90.10±1.34***
50 krad	4-6	62	90-120	1.94±0.07*** (-33.10)	3.91±0.35*** (-69.43)	3.30±0.31*** (-69.56)	0.35±0.05*** (-85.71)	89.39±1.46***
100 krad	4-6	53	90-120	1.90±0.07*** (-34.48)	3.75±0.39*** (-70.68)	2.60±0.19*** (-76.01)	0.23±0.02*** (-90.61)	91.15±0.34***
200 krad	4-6	55	90-120	1.76±0.05*** (-39.31)	2.91±0.26*** (-77.25)	2.27±0.19*** (-79.06)	0.23±0.02*** (-90.61)	89.87±1.86***
350 krad	4-6	23	90-120	1.44±0.05*** (-50.34)	1.61±0.13*** (-87.41)	1.33±0.15*** (-87.73)	0.15±0.04*** (-93.88)	88.72±0.91***
500 krad	4-6	13	90-120	1.22±0.02*** (-57.93)	1.09±0.03*** (-91.48)	1.00±0.13*** (-90.77)	0.10±0.02*** (-95.92)	90.00±1.54***
700 krad	4-6	0	-	-	-	-	-	-
LSD (p=0.05)				0.18	1.56	1.26	0.92	5.61

± S.E.

*** Significantly different from control at p=0.001. Figures in parentheses indicate percent inhibition (-) over control.

Table 26. Effect of gamma irradiated pollen on seed production in S. khasianum

Treatment	No. of seeds per berry	Filled seeds (%)	Weight of 100 seeds (mg)
Control	317.00±24.29	97.16±2.74	317.50±13.67
5 krad	258.00±22.26*** (-18.61)	48.29.1.31*** (-50.30)	124.67±26.82*** (-60.73)
25 krad	0*** (-100.00)	-	-
50 krad	0*** (-100.00)	-	-
100 krad	0*** (-100.00)	-	-
200 krad	0*** (-100.00)	-	-
350 krad	0*** (-100.00)	-	-
500 krad	0*** (-100.00)	-	-

± S.E.

*** Significantly different from control at p=0.001. Figures in parentheses indicate percent inhibition (-) over control.

Table 27. Effect of gamma irradiated pollen on berry formation in S. marginatum

Treatment	Senescence of petal after polli-nation (days)	Fruit set (%)	Fruit ripening (days)	Berry diameter (cm^3)	Berry volume (cm^3)	Berry weight (g)	Berry weight (g)	Moisture content (%)
Control	5-7	66.67	150-180	3.29±0.08	18.7±1.38	18.91±1.87	5.55±0.46	70.65±1.04
5 krad	5-7	26.32	150-180	3.08±0.19	15.26±2.99	13.96±3.51	3.03±1.27	78.30±4.12*
25-700 krad	5-7	0	-	-	-	-	-	-

± S.E.

* Significantly different from control at p=0.05.

Value in parentheses indicates percent stimulation (+) over control.

Table 28. Effect of gamma irradiated pollen on seed production in *S. marginatum*

Treatment	No. of seeds per berry	Filled seeds (%)	Weight of 100 seeds (mg)
Control	1033.00±92.09	99.68±0.75	380.00±11.24
5 krad	533.00±177.57* (-48.40)	62.85±10.62*** (-36.95)	236.94±13.82*** (-37.65)

± S.E.

* Significantly different from control at p=0.05

*** Significantly different from control at p=0.001.

Figures in parenthesis indicate percent inhibition (-) over control.

Fig. 24.1 : Effect of gamma radiation on pollen tube entry into the ovule(48 hr after pollination) of S. khasianum (All figures **x 10,000**).

a - Control
b - 5 krad
c - 25 krad
d - 50 krad
e - 100 krad
f - 350 krad (no pollen tube entered ovule)

pt - pollen tube
ov - ovule

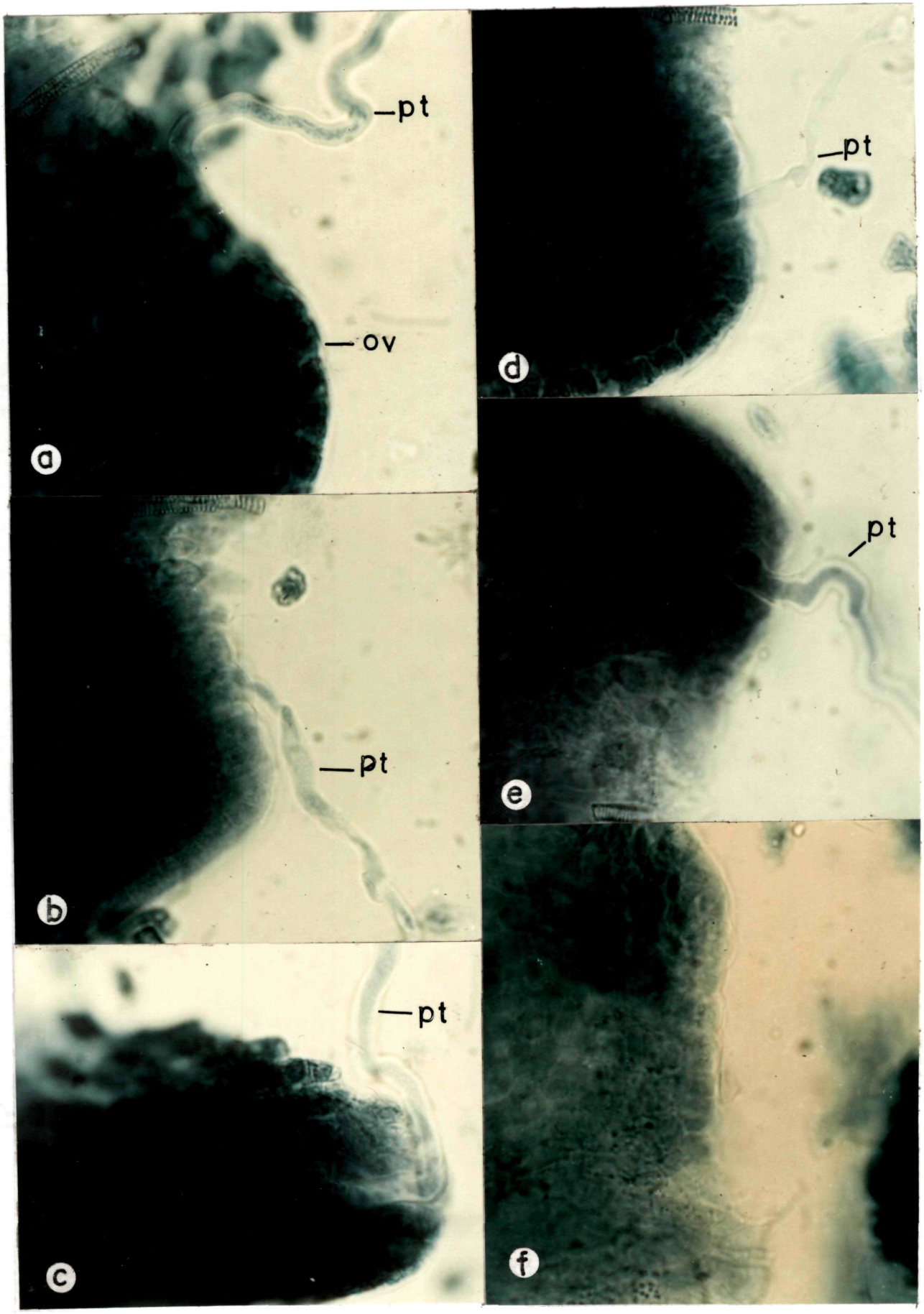


Fig. 24·1

Fig. 24.2 : Effect of gamma radiation on pollen tube entry into the ovule(96 hr after pollination) of S. khasianum (All figures **x 10,000**).

a - Control
b - 5 krad
c - 25 krad
d - 50 krad
e - 100 krad
f - 350 krad
g - 500 krad (no pollen tube entered ovule)

pt - pollen tube
ov - ovule

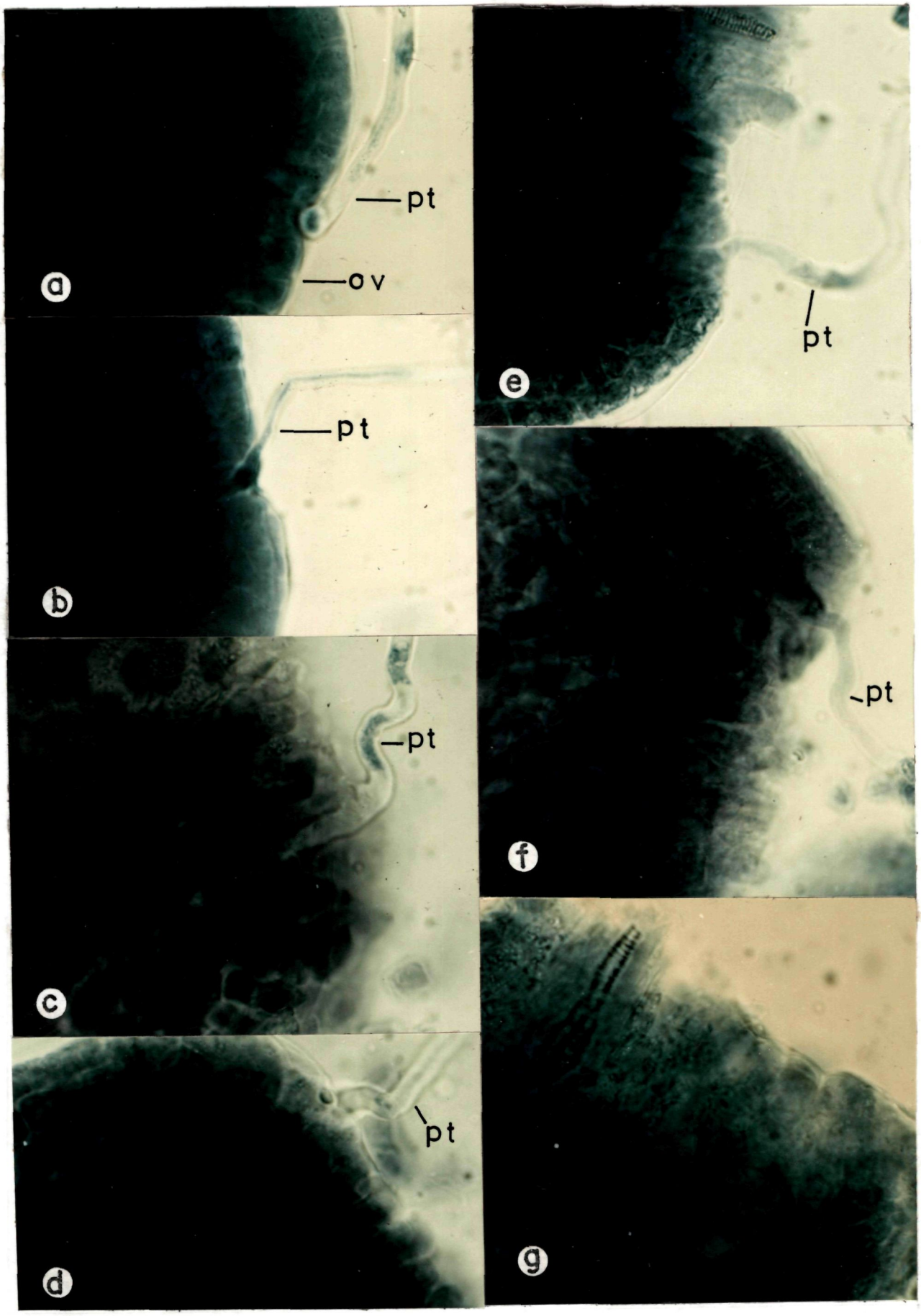


Fig.24·2

Fig. 25.1 : Cross section of ovules pollinated with various doses of gamma rays irradiated pollen, 48 hr after pollination (All figure x 6,250).

a - Control
b - 5 krad
c - 25 krad
d - 50 krad
e - 100 krad
f - 200 krad
g - 350 krad
h - 500 krad

pt - pollen tube
ea - egg apparatus
sy - synergid

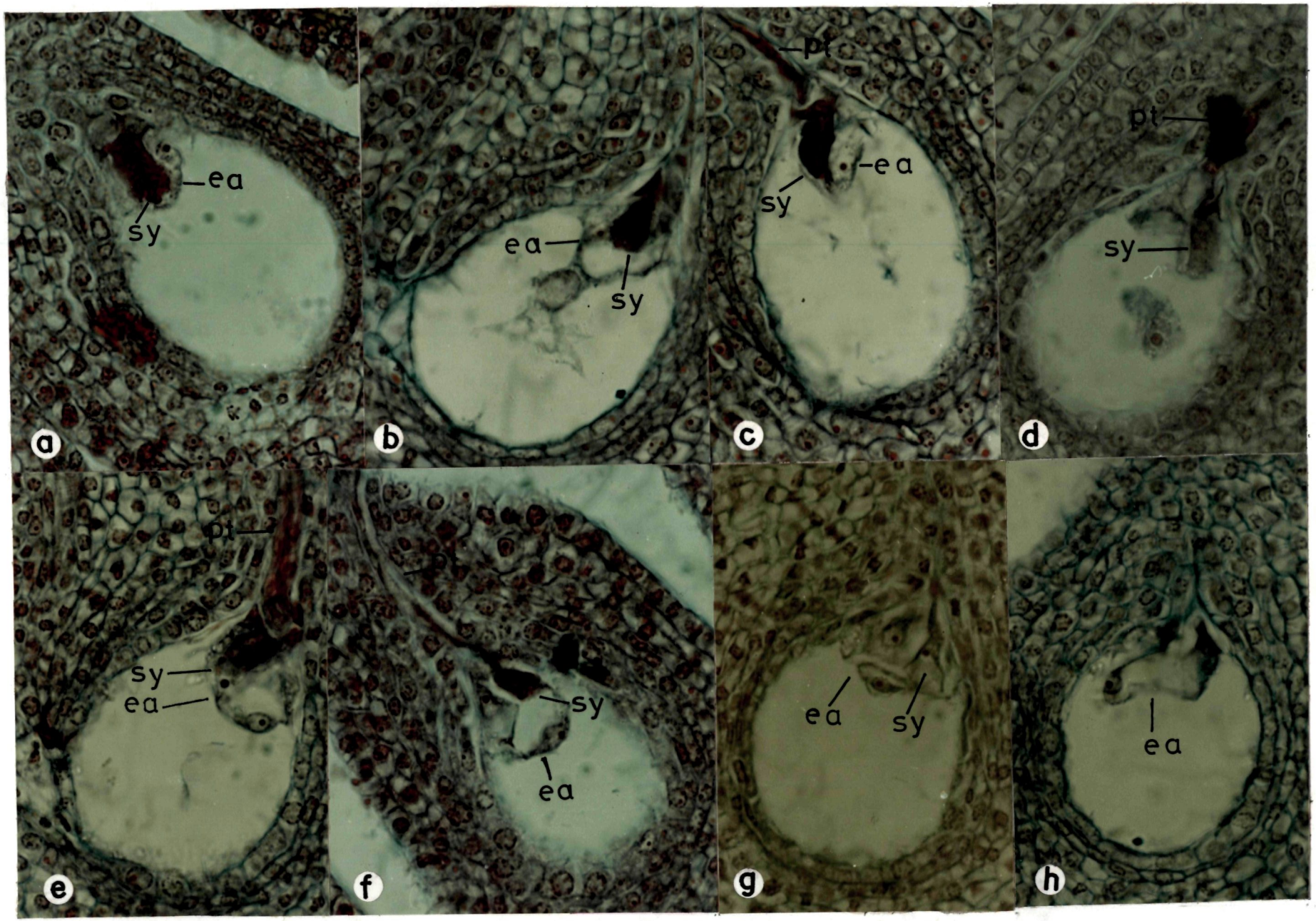


Fig.25·1

Fig. 25.2 : Cross section of ovules pollinated with various doses of gamma rays irradiated pollen, 72 hr after pollination (All figures **x 6,250**).

a - Control
b - 5 krad
c - 25 krad
d - 50 krad
e - 100 krad
f - 200 krad
g - 350 krad
h - 500 krad

pt - pollen tube
ea - egg apparatus
sn - secondary nucleus
de - degenerating egg
sy - synergid

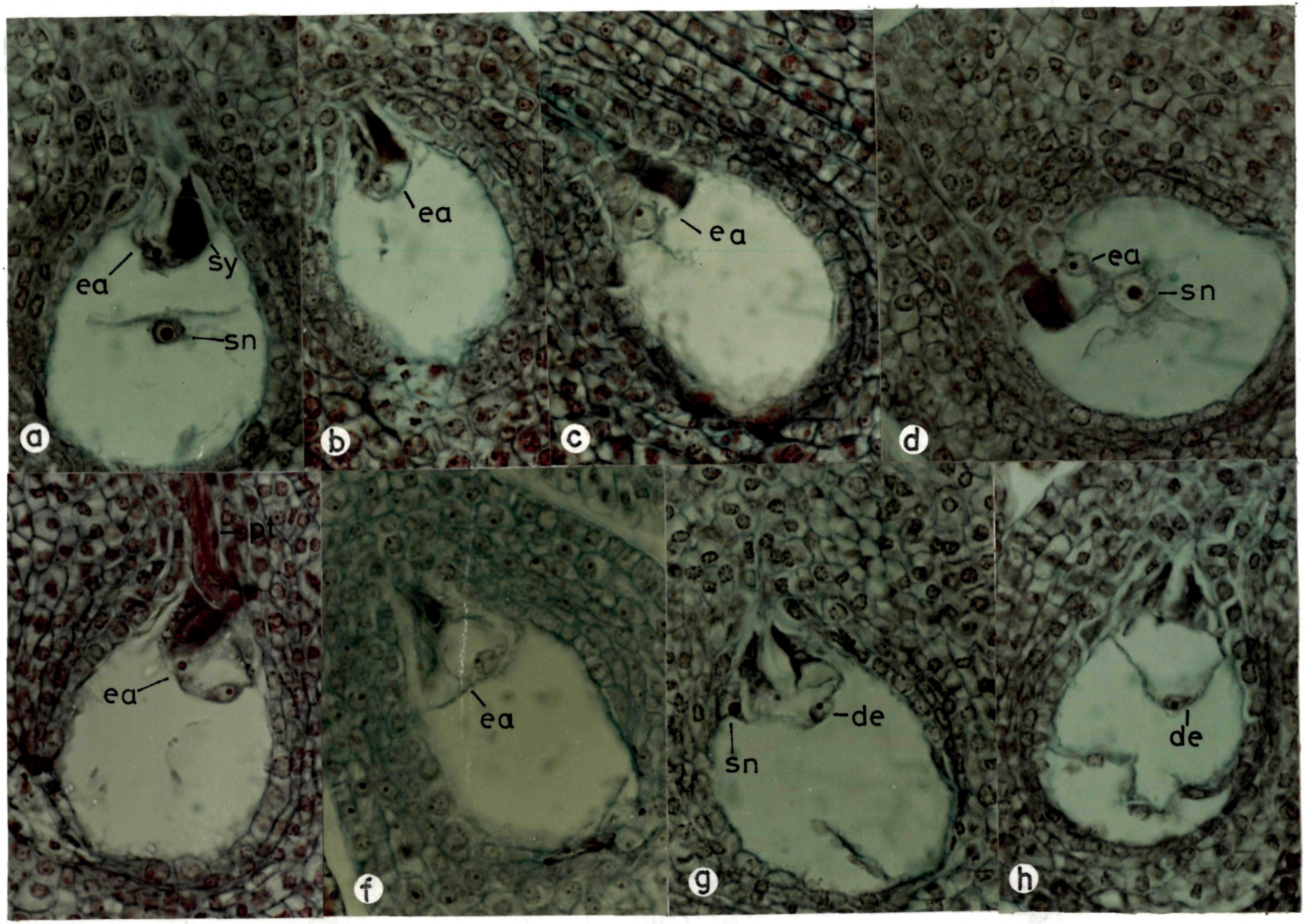


Fig. 25·2

Fig. 25.3 : Cross section of ovules pollinated with various doses of gamma rays irradiated pollen, 96 hr after pollination (All figures x 6,250).

a - Control
b - 5 krad
c - 25 krad
d - 50 krad
e - 100 krad
f - 200 krad
g - 350 krad
h - 500 krad

pt - pollen tube
ea - egg apparatus
sn - secondary nucleus
de - degenerating egg
sy - synergid

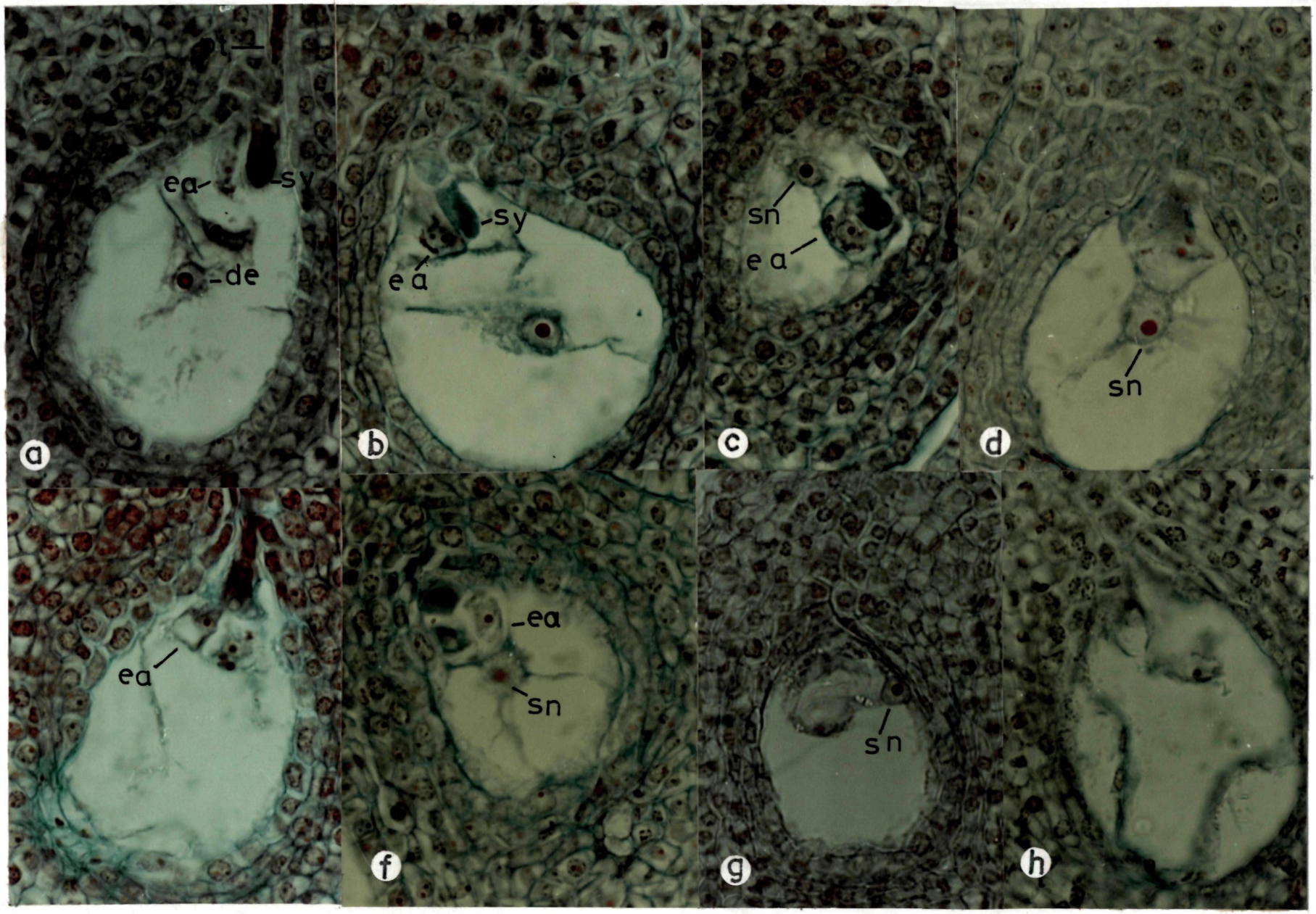


Fig. 25·3

Fig. 25.4 : Cross section of ovules pollinated with various doses of gamma rays irradiated pollen, 120 hr after pollination (All figures x 6,250).

a - Control

b - 5 krad

c - 25 krad

d - 50 krad

e - 100 krad

f - 200 krad

g - 350 krad - collapsing ovule with unfertilized enlarged egg cell

h - 500 krad - collapsing ovule with unfertilized enlarged egg cell

pe - proembryo

en - endosperm

de - degenerating egg

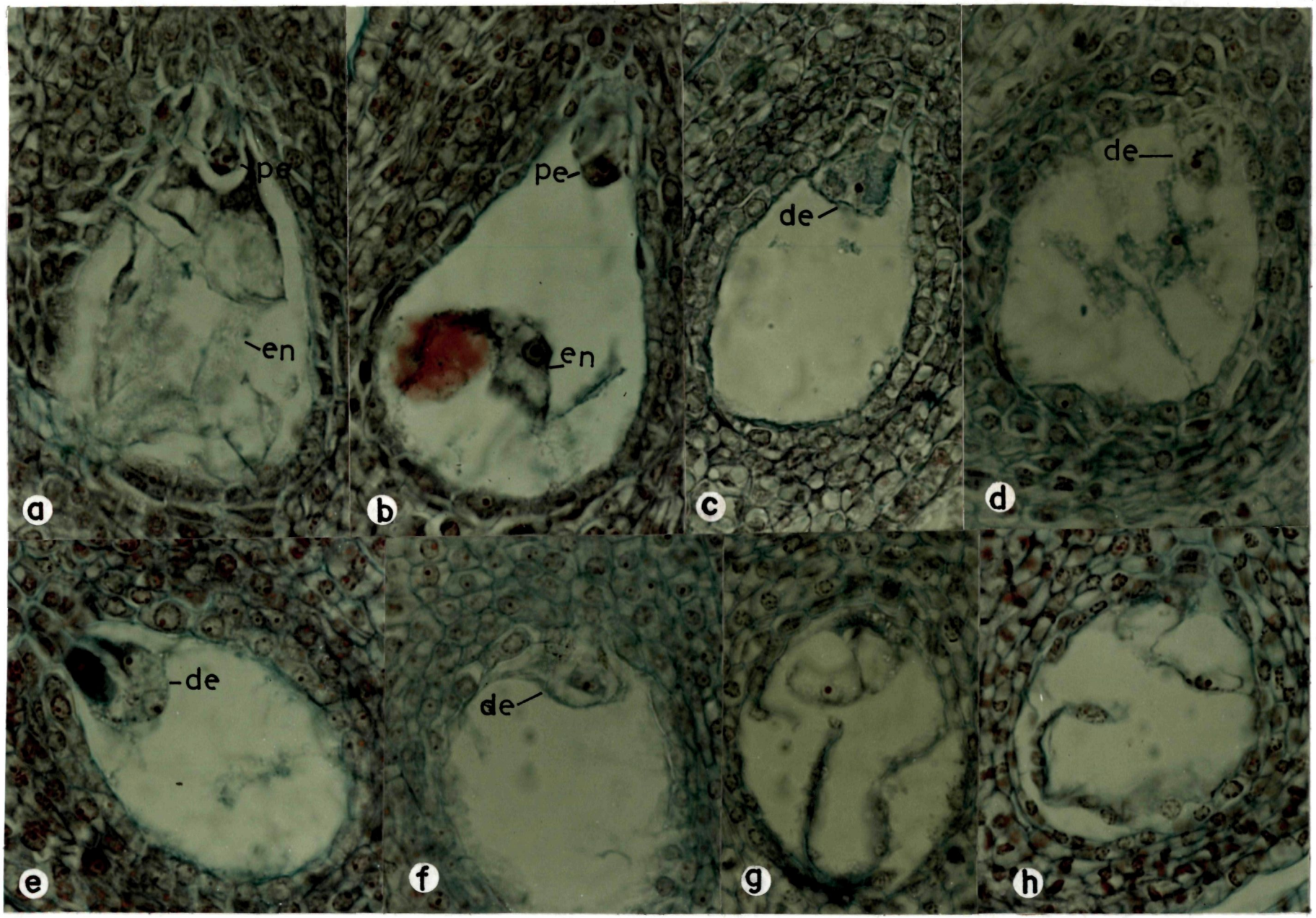


Fig.25·4

Fig. 25.5 : Cross section of ovules pollinated with various doses of gamma rays irradiated pollen, 1 week after pollination (All figures x 6,250).

a - Control
b - 5 krad
c - 25 krad
d - 50 krad
e - 100 krad
f - 200 krad
g - 350 krad - collapsing ovule with unfertilized enlarged egg cell
h - 500 krad - collapsing ovule with unfertilized enlarged egg cell

en - endosperm
de - degenerating egg
ee - enlarged endothelium
sn - secondary nucleus
dc - developing chalazal

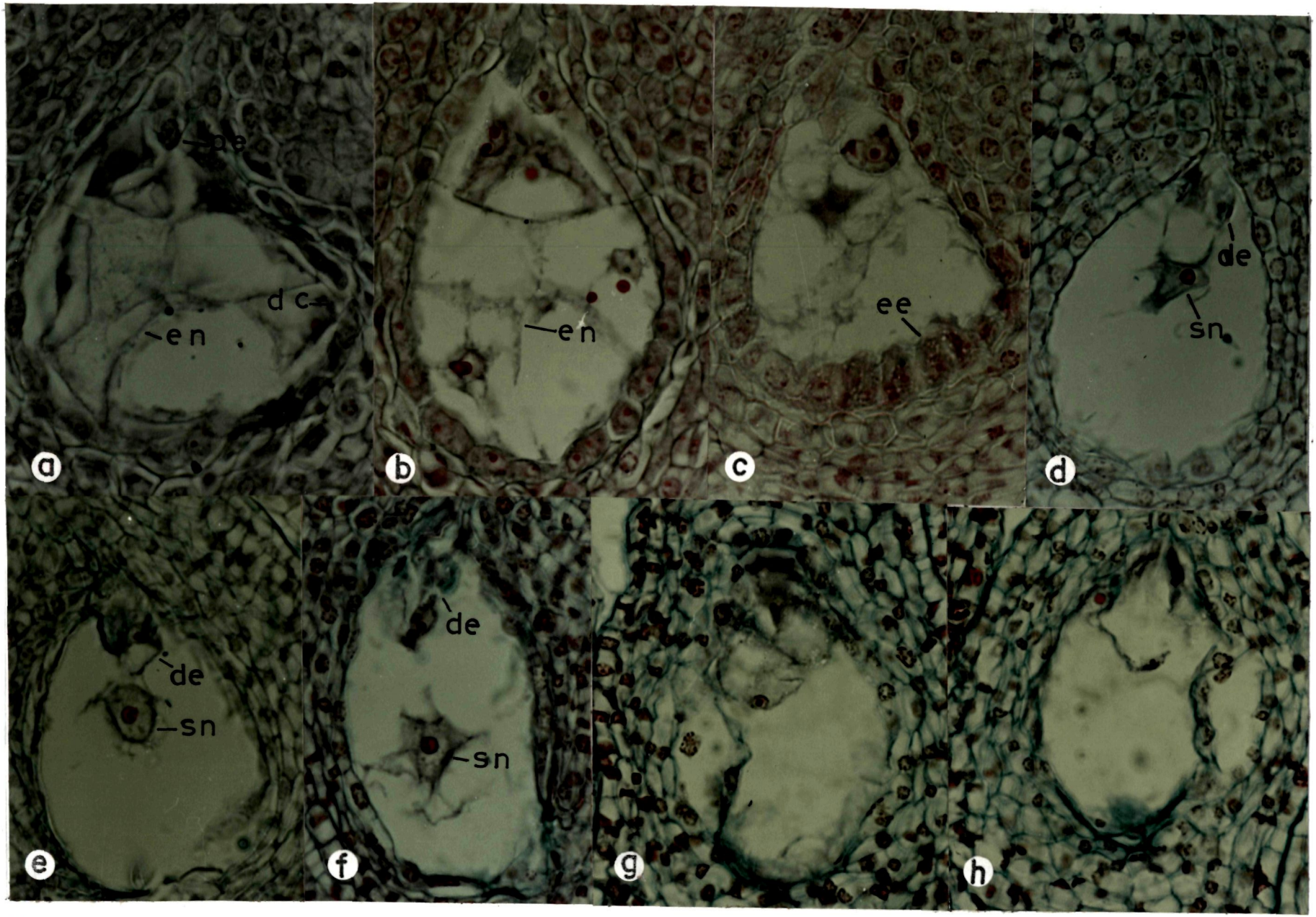


Fig.25·5

Fig. 25.6 : Cross section of ovules pollinated with various doses of gamma rays irradiated pollen, 15 days after pollination (All figures x 6,250 except b x 25,000)

a - Control
b - Control embryo magnified
c - 5 krad
d - 25 krad
e - 50 krad
f - 100 krad
g - 200 krad

em - embryo
ds - degenerating seed
pe - proliferating endothelium
ce - Chalazal end

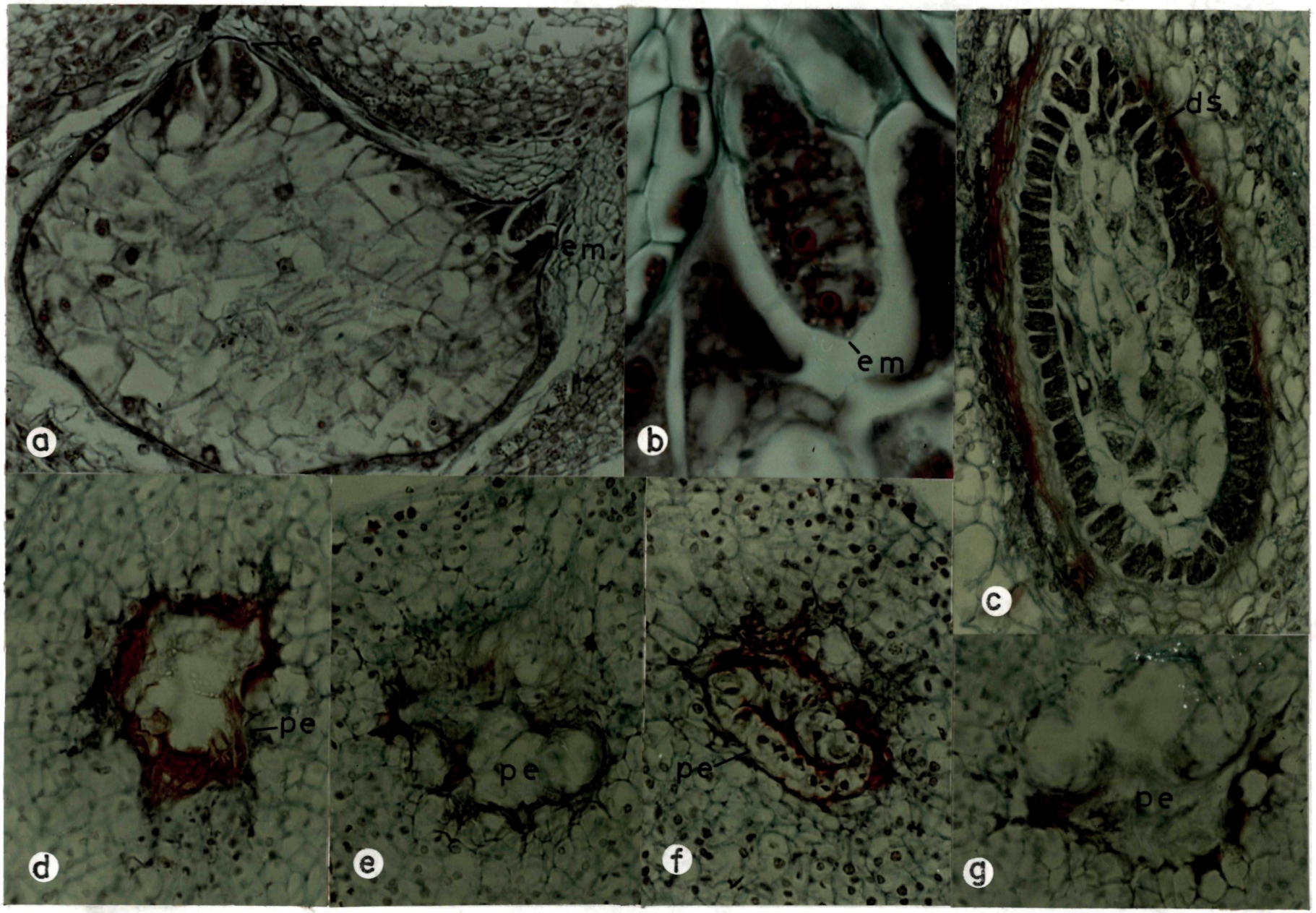


Fig.25·6

Fig. 26 : Effect of control and variously irradiated pollen on berries of *S. khasianum*.

a - Intact berries
b - Cross section of berries

1 - Control
2 - 5 krad
3 - 25 krad
4 - 50 krad
5 - 100 krad
6 - 200 krad
7 - 350 krad
8 - 500 krad

Note : Seedless berries in 3-8

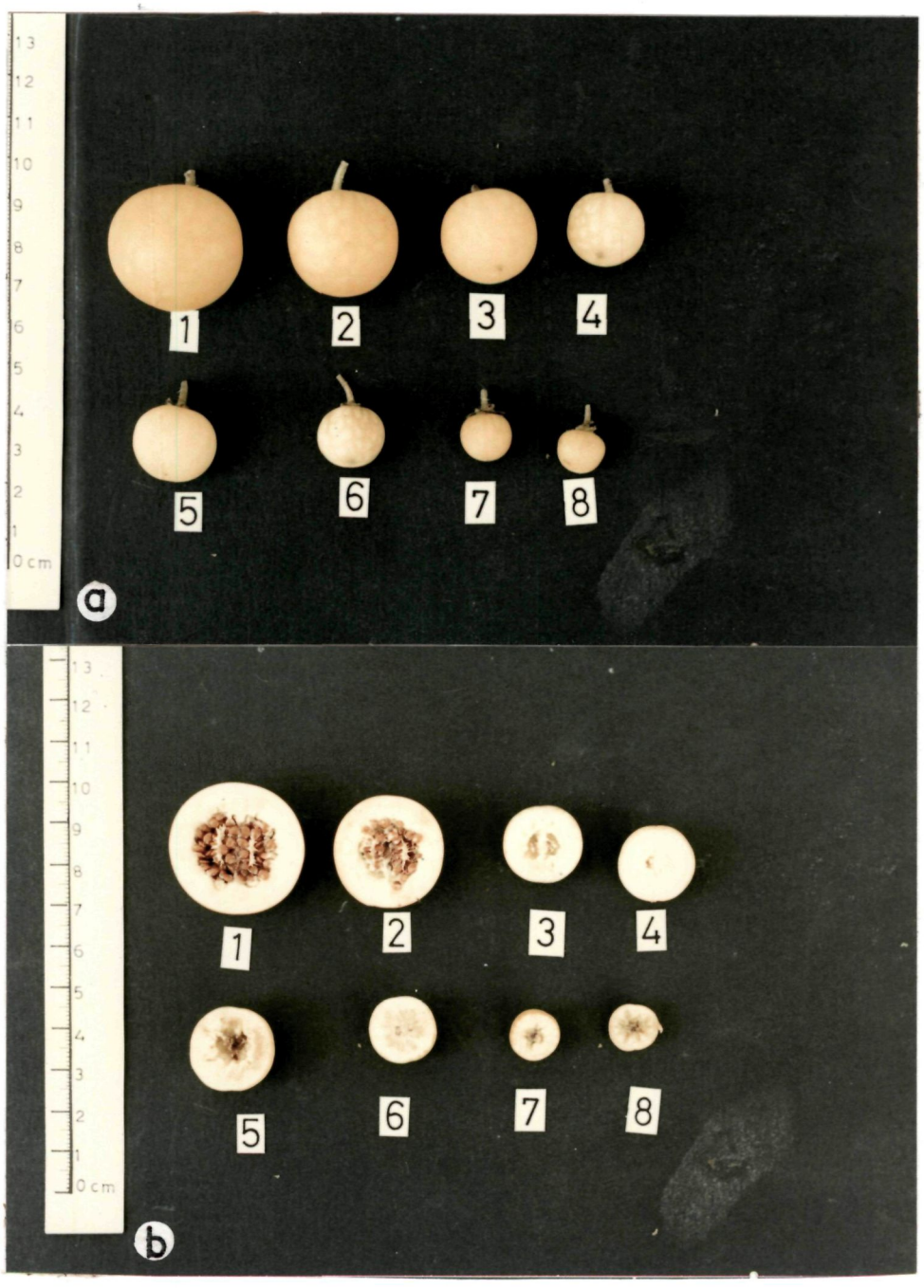


Fig. 26

Fig. 27 : Effect of gamma radiation on pollen tube entry into the ovule (48 hr after pollination) of *S. marginatum* (All figures **x 10,000**).

a - Control
b - 5 krad
c - 25 krad
d - 50 krad
e - 100 krad
f - 350 krad - no pollen tube entered ovule

pt - pollen tube
ov - ovule

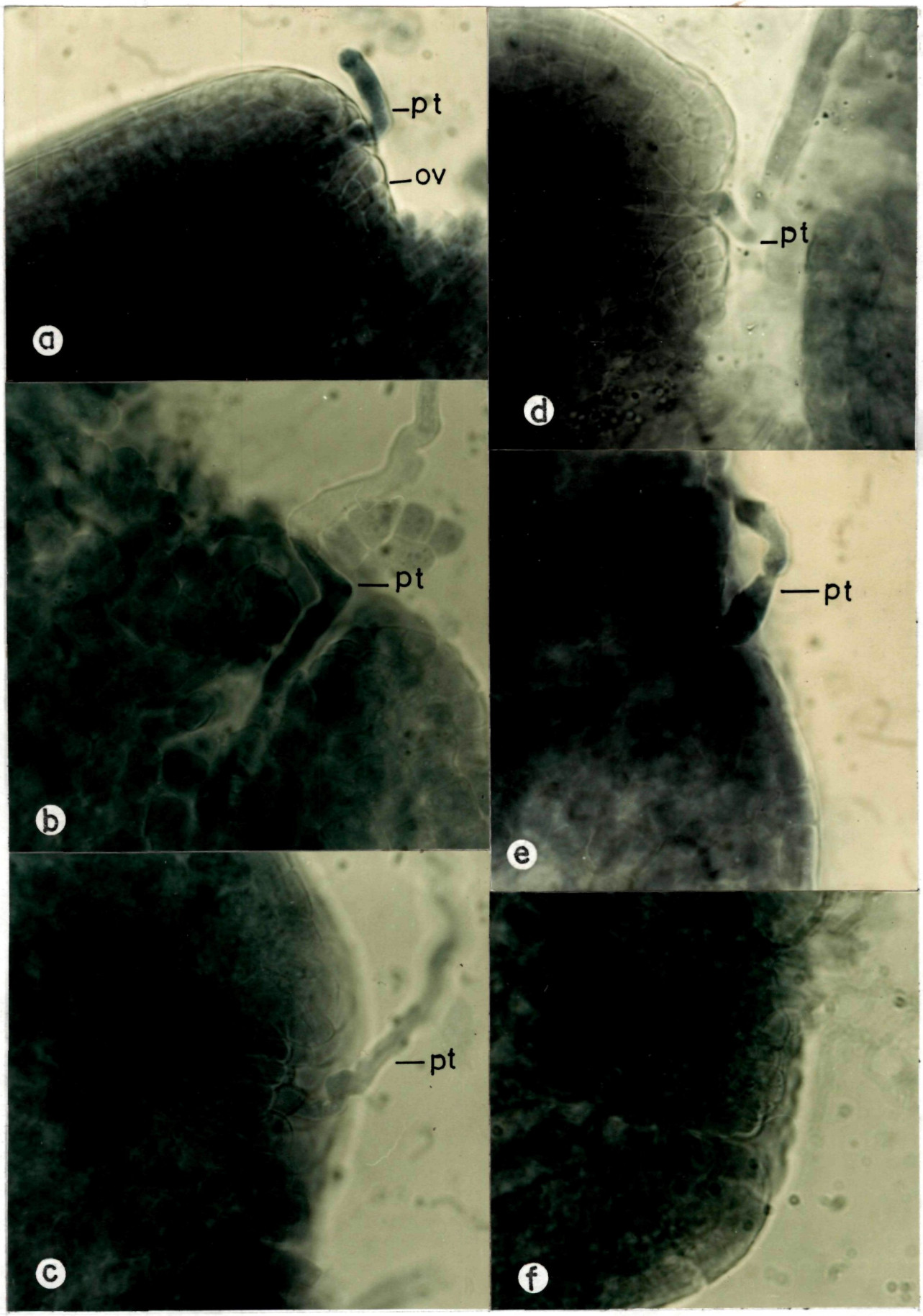


Fig. 27

Fig. 28.1 : Cross section of S. marginatum ovules pollinated with various doses of gamma rays irradiated pollen at different time intervals (All figures x 6,250).

a - ovule pollinated with control pollen, 48 hr after pollination

b - ovule pollinated with 25 krad irradiated pollen 48 hr after pollination

c - ovule pollinated with 50 krad irradiated pollen 48 hr after pollination

d - ovule pollinated with control pollen 96 hr after pollination

e - ovule pollinated with 25 krad irradiated pollen 96 hr after pollination

f - ovule pollinated with 50 krad irradiated pollen 96 hr after pollination

pt - pollen tube

sy - synergid

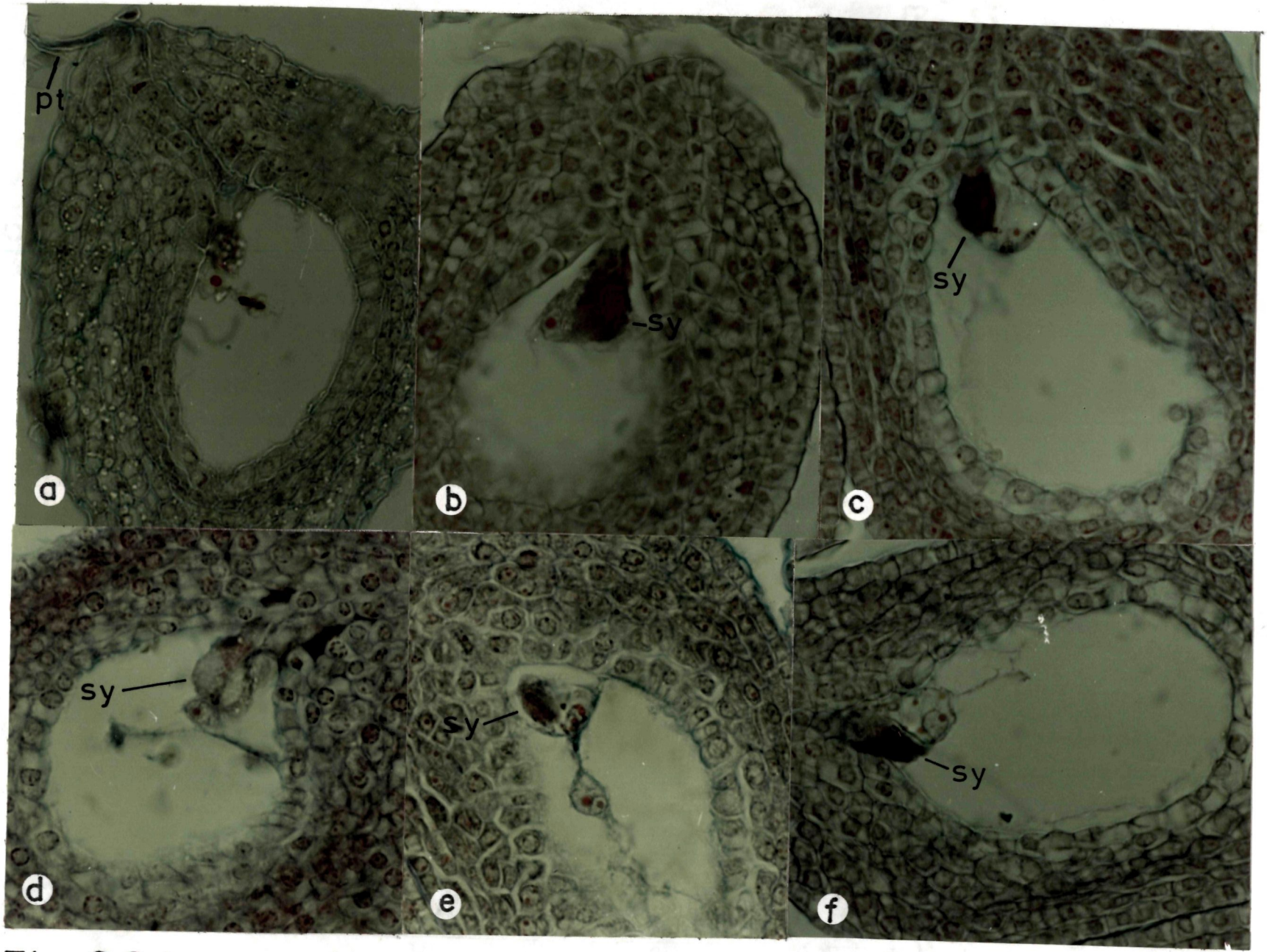


Fig 28.1

Fig. 28.2 : Cross section of S. marginatum ovules pollinated with various doses of gamma rays irradiated pollen at different time intervals (All figures x 6,250).

a - ovule pollinated with control pollen 120 hr after pollination

b - ovule pollinated with 25 krad irradiated pollen 120 hr after pollination

c - ovule pollinated with 50 krad irradiated pollen 120 hr after pollination

d - ovule pollinated with control pollen 1 week after pollination

e - ovule pollinated with 25 krad irradiated pollen 1 week after pollination

f - ovule pollinated with 50 krad irradiated pollen 1 week after pollination

zy - zygote

de - degenerating egg

en - endospermic nucleus

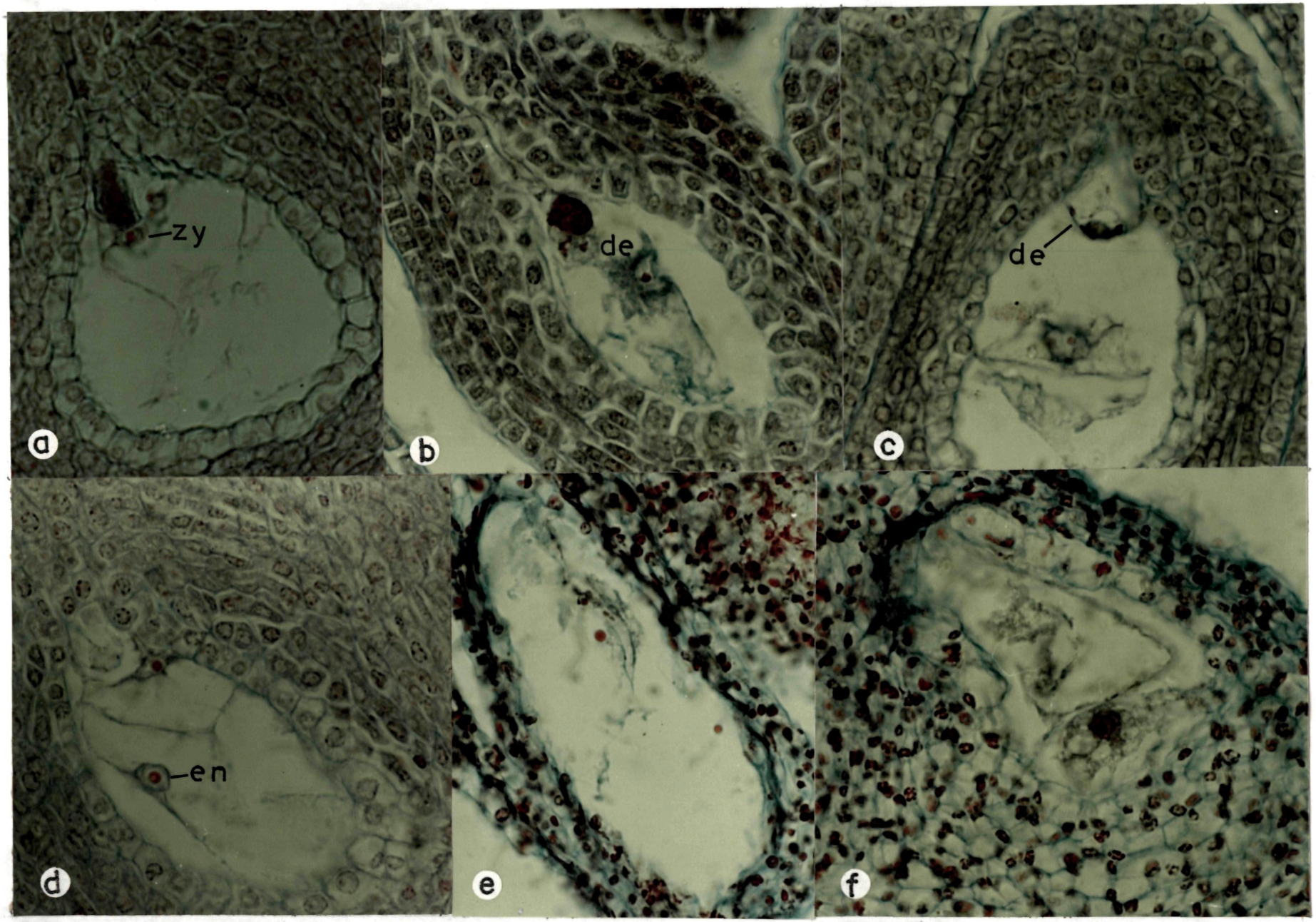


Fig. 28·2

Fig. 29 : Effect of control and irradiated pollen on berries of *S. marginatum*.

1 - control berry

2 - berry formed from pollen irradiated with 5 krad gamma rays.

Fig. 29

Chapter-V

M_1 GENERATION OF S. KHASIANUM

INTRODUCTION

Germination in seeds formed due to irradiated pollen is greatly affected. The use of irradiated pollen increase the frequency of cotyledonary abnormalities (Devreux and Mugnozza, 1964), and induce increased germinant variability (Clausen, 1973b). The most common features of aberrant phenotypes are dwarfs, variegated leaves, reduced flower size, aborted flowers and pollen sterility (Brewbaker and Emery, 1962).

The primary advantage of pollen irradiation is that F_1 plants are rarely, if ever, chimeral, while the F_1 plants from irradiated seed are inevitably and grossly so

(Brewbaker and Emery, 1962). For both the critical radio-botanical researches and the mutation breeding approach, such chimeras are headache, if not a down right anathema (Brewbaker and Emery, 1962).

Although mutations induced by pollen irradiated with ionizing radiations have been used to the advantage of plant breeders in many plants, its utility in improving Solanum khasianum and S. marginatum, two glycoalkaloid yielding plants, has not been investigated so far. Therefore, the present research work was undertaken to investigate the effects of gamma irradiated pollen on the M_1 plants of S. khasianum. Following characters were taken into consideration in the present investigations :

1) Seed germination
2) Seedling growth abnormalities
3) Photosynthetic pigments
4) Flowering and Reproduction

MATERIAL AND METHODS

Seed Germination

Seeds of S. khasianum collected from berries of control and berries formed due to 5 krad irradiated pollen (5 krad berries) were sown towards the end of January, 1989, in buckets (20 x 21 cm) filled with a mixture of

soil and manure (1:1). Three replicates consisting of 100 seeds per pot were maintained. The pots were watered at regular intervals to keep the soil moist. These pots were kept in experimental net house. Another set of experiment using 100 seeds each of control and 5 krad berries were soaked in sterilized water and spread in sterilized petri plates having moist cotton and filter paper. The petri plates were incubated in a B.O.D. incubator maintained at 25 ± 1°C. In both the experiments the number of seeds germinated were recorded at regular intervals upto 90 days. Percentage seed germination was calculated and statistical calculations for finding out significant differences, were made using angular transformation values.

Seedling Growth and Abnormalities

Abnormalities in seedlings, obtained from seeds produced using 5 krad irradiated pollen (5 krad plants), were observed. Growth and morphology of these seedlings were compared with the control seedlings. Initially, the number of cotyledons were taken into consideration. Seedlings having two leaves were transferred into pots (20 x 21 cm) filled with 1:1 soil and manure; per pot only one seedling was maintained. Later when the seedlings produced 4-5 leaves, these were scrutinized for leaf abnormalities. Subsequent observations were recorded from ten plants per treatment

(control and 5 krad plants). Plant height; plastochron index; size of the fully expanded 6th leaf; number of spines and spine frequency were recorded in 6 week old control and 5 krad plants using the following formulae :

Plastochron Index (Maksymowych, 1973) :

$$PI = n + \frac{LogL_n - Log10}{LogL_n - LogL_{n+1}}$$

where n = the serial number of that leaf (counting from base) which is just longer than 10 mm.

$LogL_n$ = the logarithm of length (mm) of leaf 'n', which is longer than 10 mm.

$LogL_{n+1}$ = the logarithm of length of leaf n+1 which is just shorter than 10 mm.

Spine Frequency

$$\text{Spine frequency} = \frac{\text{Total number of spines}}{\text{Area of leaf}}$$

Later various 5 krad plants were classified considering the number of cotyledons and leaf characters. In these plants average size of the leaf, number of spines, spine frequency and angle of spine were determined scrutinizing 8th-11th leaves. When these plants were fully mature, plant height spread and number of branches were also recorded.

Photosynthetic Pigments

Leaf pigments such as Chlorophyll 'a', Chlorophyll 'b' and carotenoids were estimated in control and 5 krad plants showing colour variations following the methods given by Mahadevan and Sridhar (1982) :

Chlorophylls

1 gm of fresh leaves were cut into small pieces and homogenised with excess acetone, in a mortar with pestle. It was decanted and the supernatant was filtered using Whatman No. 42 filter paper. Sufficient quantity of 80% acetone was added and extraction was repeated. The residue was washed several times with acetone till the filtrate became colourless. The filtrates were then pooled together and the volume was made upto 100 ml in a volumetric flask. 5 ml of this filtrate was further diluted to 50 ml with 80% acetone. The absorbance of this extract was measured at 645 and 663 nm using Systronics Spectrophotometer for determination of chlorophyll 'a' and 'b'. The absorbance of the extract was also measured at 652 nm for total Chlorophyll. The concentration of total Chlorophylls and Chlorophyll 'a' and 'b' were calculated using specific absorption coefficients.

1. A_{663} = 82.04 Ca + 9.27 Cb

2. A_{645} = 16.75 Ca + 45.60 Cb

where A is the absorbance

Ca = Chlorophyll 'a'

Cb = Chlorophyll 'b'

Total Chlorophyll (mg/l) = $20.20\ A_{645} + 8.02\ A_{663}$

Chlorophyll 'a' (mg/l) = $12.7\ A_{663} - 2.69\ A_{645}$

Chlorophyll 'b' (mg/l) = $22.9\ A_{645} - 4.68\ A_{663}$

Total Chlorophyll was also calculated using the formula :

$$\text{Total Chlorophyll (mg/l)} = \frac{1000\ A_{652}}{36} \text{ or } 27.8\ A_{652}$$

The Chlorophyll content on fresh weight basis was calculated using the following equations :

$$\text{Total Chlorophyll (mg/l)} = \frac{20.2\ A_{645} + 8.02\ A_{663}}{a + 1000 \times W} \times V$$

$$\text{Chlorophyll 'a' (mg/l)} = \frac{12.7\ A_{663} - 2.69\ A_{645}}{a \times 1000 \times W} \times V$$

$$\text{Chlorophyll 'b' (mg/l)} = \frac{22.9\ A_{645} - 4.86\ A_{663}}{a \times 1000 \times W} \times V$$

where a = length of light path in the cell (=1 cm)

V = volume of the extract in ml

W = fresh weight of leaf in g.

Carotenoids

2 g of freshly collected leaf was finely cut and homogenized with 20 ml of acetone using mortar and pestle. The extract was decanted and filtered through Whatman No.42. Extraction was repeated till the filtrate became colourless. The extracts were pooled together and

partitioned thrice by adding equal amount of ether using a separator funnel. Water was added to produce two layers during the initial ether extraction. The ether phase contained carotenoids. Ether extracts were combined and evaporated on a hot water bath at 35°C. The residue was dissolved in 10 ml of ethanol, and 1 ml of 60% aqueous KOH solution was added to saponify it. This process removes chlorophylls and interfering lipids and also cleaves the esterified carotenoids. This mixture was boiled for 5-10 min and left overnight at room temperature. Equal amount of distilled water was added to this and partitioned twice with ether. This sollution was evaporated and redissolved in 50 ml ethanol. The absorbance of this solution was measured at 450 and 670 nm using Systronics Spectrophotometer. Since the value of absorbance at 450 nm is 10 times greater than that at 670 nm carotenoids present in this extract was estimated as follows :

$$\text{Total carotenoids (mg)} = \frac{D \times V \times f \times 10}{2500}$$

where D = absorbance value at 450 nm in 1 cm cell

V = volume of the original extract in ml

f = dilution factor

2500 = average extinction coefficient.

For both chlorophylls and carotenoids estimation three replicates were maintained.

Flowering and Reproduction

The selected control and 5 krad plants were allowed to flower and during this period flower size, number of flowers per cluster, number of long-styled flowers per cluster, long-styled flower frequency, number of sepals, petals and stamens were recorded.

Pollen viability of four selected 5 krad plants were tested and compared with control. For this purpose acetocarmine method was used. For preparing acetocarmine solution 1% carmine stain was used (55 ml of distilled water, 45 ml of acetic acid and 1 g of carmine stain powder were mixed and boiled gently for 5 min, cooled and filtered). A drop of acetocarmine solution was placed on a clean glass slide. Pollen grains from just anthesized flowers were carefully taken out with the help of a needle and placed in 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 were observed under Olympus research microscope and total number of viable and non-viable pollen grains were counted from 5 microscopic fields. Viable pollen grains stained deep red while non-viable pollen grains did not stain. At least 5 anthers were taken for each plant and stained separately.

Except in control and 5 krad normal leaf type, all other 5 krad plants produced very less quantity of pollen grains. Therefore, collection of pollen grains from these plants was difficult and thus pollen germination test could not be performed.

Berry forming ability of control and 5 krad plants as also fertilizing ability of pollen of 5 krad plants was assessed by hand pollinating emasculated flowers with self and control pollen. The method of emasculation and pollination was as described in Chapter IV. Some of the flowers were allowed to remain undisturbed to assess the fruit formation in natural condition. The response was assigned + (fertile) or - (sterile) accordingly.

The berries formed in control and all the 5 krad phenotypes were allowed to ripen. The number of berries developed per flower cluster and per plant was recorded. At maturity the berries were harvested and berry size, fresh and dry weight, moisture content, number of seeds per berry and weight of 100 seeds were also recorded using the methods described in Chapter IV.

RESULTS

Seed Germination

Table 29 gives data on germination of seeds (collected from control and 5 krad berries of S. kha[illegible]ianum)

both *in vitro* and in pots. Seeds of both control and 5 krad berries germinated within 20 days of incubation under *in vitro* condition in B.O.D. incubator at 25 ± 1°C. On the 20th day of incubation in control though 17% of the seeds germinated only 5% germination was evident in 5 krad seeds (seeds of 5 krad berries) **(Table 29)**. At the end of 100 days compared to 95% seed germination in control only 43% 5 krad seeds germinated **(Table 29)**. In control seeds germination was completed in 50 days but 5 krad seeds germinated upto 60th day **(Table 29)**. Unlike *in vitro* seed germination, the germination of both control and 5 krad seeds in pots occurred only on the 50th day of sowing. On this day 36.67% of control seeds and 11.67% of 5 krad seeds germinated **(Table 29)**. On the 90th day, 93.33% of control seeds and 43% of 5 krad seeds had germinated **(Table 29)**. Seed germination in control seeds was over on the 80th day of sowing but in 5 krad seeds it continued till 90th day of sowing **(Table 29)**. It is evident from the **Table 29** that seed germination was greatly impaired in seeds of berries produced due to irradiated pollen.

Seedling Growth and Abnormalities

Table 30 gives data on the cotyledonary seedling abnormalities in seedlings produced by control and 5 krad seeds. 280 seedlings of control and 129 seedlings of 5 krad seeds were scrutinized for seedling abnormalities.

No seedling abnormality was observed in control seedlings (**Figs. 30a,c & 31 Cont.**). However, 10 seedlings (7.75%) developed from 5 krad seeds (5 krad seedlings) were abnormal (**Table 30**). The abnormalities observed revealed development of varying number of cotyledons and their fusion (**Fig. 30b**) and leaves of abnormal shapes (**Figs. 30d & e**). Seedlings of 5 krad seeds were thus categorised into the following abnormalities; dicotyledon seedlings having triangular leaves (**Figs. 30d & 31b**); dicotyledon seedlings having roundish leaves (**Figs. 30e & 31c**) and tricotyledonous seedlings in which all the cotyledons were fused together at the base (**Fig. 30b**).

Table 31 gives data on plant height, plastochron index, leaf size, number of spines and frequency of spines on the 6th leaf of control and 5 krad plants of diverse phenotypes. Six week old control and diverse 5 krad plants had no difference in height, plastochron index and number of spines on the 6th leaf (**Table 31**). Leaf size and spine frequency in phenotype I, II and IV of 5 krad plants though did not differ from control (**Table 31**), leaf length; breadth and leaf size in phenotype III of 5 krad plant was reduced significantly (**Table 31**). The spine intensity of leaves of 5 krad phenotype III was more than control (**Table 31**).

Data on average length, breadth and area of leaves, average number of spines on leaves, spine frequency length of spine and angle of spine in control **(Fig.32a)** and various phenotypes of 5 krad plants : dicotyledon with normal leaf type (Phenotype I) **(Fig. 32b)**; dicotyledon triangular leaf type (Phenotype II) **(Fig. 32c)**; dicotyledon roundish leaf type (Phenotype III) **(Fig. 32d)** and tricotyledon in which all the cotyledons were fused together at the base (Phenotype IV) **(Fig. 32e)** are given in **Table 32.** Leaf size in control and 5 krad plants of phenotype I did not differ but 5 krad plants of all other phenotypes (II, III and IV) had much smaller leaves compared to control **(Table 32).** Average number of spines on the leaves in 5 krad phenotype II plant was significantly less than control but number of spines in other types of 5 krad plants did not differ significantly from control **(Table 32).** Whereas phenotypes III and IV were more spiny than control, phenotypes I and II resembled control in spininess **(Table 32).** Spines in phenotype IV of 5 krad plants were significantly smaller than spines in control plants. In phenotype II spines though size of the spines on the lower surface of leaf was significantly reduced, the size of the spines on the upper surface of leaf did not differ from control **(Table 32).** Spine length in other two categories (phenotypes I and III) of 5 krad plants was similar to control. Compared to control, reduced angle of spine was

evident in 5 krad phenotype II (**Table 32**). In all other categories of 5 krad plants spine angle did not differ from control (**Table 32**).

Photosynthetic Pigments

Visual observation of M_1 plants suggested that leaves of certain phenotypes of 5 krad plants were either more green or less green in comparison to control leaves. Therefore, a comparison of photosynthetic pigments of these plants and control was made. Comparative data on chlorophyll 'a', chlorophyll 'b', total chlorophyll and carotenoids of control and phenotypes I-III are given in **Table 33**. The analysis revealed that while 5 krad phenotype I leaves (**Fig. 31a**) had lesser amount of chlorophyll 'a' and 'b', the leaves of 5 krad phenotype III (**Fig. 31c**) contained higher amount of chlorophyll 'a', however, the amount of chlorophyll 'b' remained the same as control (**Table 33**). **Table 33** also reveals that total chlorophyll in the leaves of 5 krad phenotype I was less than control but phenotype II leaves were richer in chlorophyll content vis-a-vis control. The chlorophyll content of leaves of 5 krad phenotype III plant did not differ from control (**Table 33**). Compared to control significant increase occurred in carotenoids content of leaves of all the three phenotypes (I, II and III) of 5 krad plants (**Table 33**). Maximum amount of carotenoids was

extracted in 5 krad type II plant **(Table 33).**

Flowering and Reproduction

Data on number of flower cluster per plant, total number of flowers per plant, number of flowers per cluster, total number of long-styled flowers per plant, number of long-styled flowers per cluster, and long-styled flower frequency in control and 5 krad plants of diverse phenotypes are given in **Table 34.** Control plants produced 15 flower clusters per plant in pots under net house condition. In 5 krad phenotype II the number of flower clusters was significantly increased to 61 per plant **(Table 34).** Total flower clusters per plant in all other categories of 5 krad plants did not differ from control **(Table 34).** Control plants produced an average of 53 flowers per plant while 5 krad phenotype II plant produced a total of 354 flowers; an increase of 567.92% over control **(Table 34).** In all other phenotypes of 5 krad plants number of flowers produced by each plant was not different from control **(Table 34).** It is evident from **Table 34** that maximum number of flowers was produced by 5 krad phenotype II plant. In this plant 3-11 flowers were produced in a cluster; average per cluster being 5.74. On the other hand, number of flowers per cluster in 5 krad phenotype III was significantly less than control **(Table 34).** Total number of long-styled flowers per plant was

also significantly increased over control in plants of 5 krad phenotypes II and IV **(Table 34).** Maximum number of long-styled flowers (290) per plant was produced by phenotype II plant compared to 18.67 in control. Similarly, number of long-styled flowers in 5 krad phenotype IV plants was also significantly more than control **(Table 34).** Control plants produced 1-4 long-styled flowers per cluster (mean 1.28) while 5 krad phenotype II plant produced 2-11 long-styled flowers (mean 4.75) per cluster. Similarly, number of long-styled flowers per cluster was significantly increased to 2.53 and 4.33 in 5 krad phenotype III and IV plants, respectively **(Table 34).** Long-styled flower frequency was significantly enhanced in 5 krad phenotypes II, III and IV plants **(Table 34).** Maximum long-styled flower frequency (100%) was observed in 5 krad phenotype IV plant which was followed by phenotype III (97.44%) and phenotype II (82.75%) **(Table 34).**

Variations in floral morphology and pollen stainability in control and various categories of 5 krad plants are given in **Table 35.** Control flowers measured 10.60 mm while the flower size was reduced significantly to 9.50 and 7.30 mm in 5 krad phenotypes II and III plants respectively **(Table 35).** Normal (control) flower produced 5 sepals, 5 petals and 5 stamens **(Fig. 33a).** However, in the present study 5 krad plants produced variable number

of sepals (4-6), petals (4-6) and stamens (3-7) (Table 35; Figs. 33b-g). Other abnormalities observed in the flowers of various 5 krad plants included abnormal development of stamens (Fig. 33c), broad stigma (Fig. 33d), splitted stigma (Fig. 33e), modification of stamen into pistil like structure (Fig. 33f) and fusion of stamen with the pistil (Fig. 33g). Compared to control though the anther size in 5 krad phenotype II and III plants was significantly reduced, anthers in 5 krad phenotype IV plant were significantly bigger in relation to control anthers (Table 35). Table 35 also gives comparative data on the stainability of pollen in control and 5 krad plants of diverse phenotypes. Over 90% of control pollen stained with acetocarmine (Fig. 34a). The ability of pollen to stain with acetocarmine was, although greatly reduced in 5 krad phenotypes II (Fig. 34c), III (Fig. 34d) and IV (Fig. 34e), the pollen of 5 krad phenotype I plants did not differ from control pollen in their ability to stain (Table 35; Fig. 34b).

Table 36 gives data on the berry forming ability of control and 5 krad plants of diverse phenotypes. All the test plants developed berries under natural condition (open pollination) (Table 36). Berries also developed when the flowers were hand pollinated with control pollen (Table 36). But when the flowers of control and 5 krad plants of diverse categories were hand pollinated with

self pollen only control and 5 krad plants of phenotype I formed berries. The other categories (Phenotypes II, III and IV) of 5 krad plants failed to develop berry **(Table 36)**. These results suggest that the pistil in all the test plants was fertile (+). Further, pollen of control and phenotype I of 5 krad plants were although fertile, the pollen of phenotype II, III and IV of 5 krad plants were sterile(-) **(Table 36)**.

Table 37 gives data on plant height, spread, number of branches, total berry per plant and number of berry per cluster in control and various phenotypes of 5 krad plants at maturity. Compared to control 5 krad plants of phenotypes II and III were less tall but other categories of 5 krad plants (Phenotypes I and IV) were similar to control plants in their height **(Table 37)**. In comparison to control, spread and number of branches produced by phenotypes II, III and IV were significantly higher **(Table 37)**. The number of berries produced per plant was significantly less than control in phenotype III, but in phenotype IV plants it was significantly more than the berries in control plants **(Table 37)**. There was no significant difference between control and various phenotypes of 5 krad plants in the number of berries per cluster **(Table 37; Figs. 35a-e)**.

Data on berry size, fresh and dry weight of berry, moisture content, number of seeds per berry and weight of

seeds in control and various categories of 5 krad plants are given in **Table 38.** Compared to control berry **(Figs. 36.1,2)**, berry size, fresh and dry weight of berry were greatly reduced in 5 krad phenotype II, III and IV plants **(Table 38; Figs. 36.6-36.8).** However, moisture content in berries of these 5 krad phenotypes (II, III and IV) was significantly increased **(Table 38).** Number of seeds produced by each berry in 5 krad plants of phenotypes II, III and IV was also reduced significantly **(Table 38).** Berries of phenotype I of 5 krad plants **(Figs. 36.3,4)** did not differ from control in berry size, fresh and dry weight, moisture content and number of seeds per berry **(Table 38).** Seeds of all the categories of 5 krad plants resembled control seeds in their weight **(Table 38).**

In the present research work, content of total glycoalkaloid/solasodine in berries of various phenotypes of 5 krad plants could not be determined due to insufficient quantity of dried berries.

DISCUSSION

Comparison of germination of seeds from control and seeds formed due to 5 krad irradiated pollen in S. khasianum revealed that seed germination was greatly reduced in the latter under both in vitro and in vivo condition. The rate of germination, compared to control,

was also reduced in seeds produced by irradiated pollen. Reduced germination of seeds formed due to irradiated pollen is also reported in Betula nigra (Clausen, 1973a), rice (Chin and Gordon, 1989) and date palm (Kgazal, 1989).

The present study revealed that seedling abnormalities were induced in germinants from seeds formed due to irradiated pollen. Morphological abnormalities in these seedlings included change in number of cotyledons, different degree of cotyledonary fusion and varying leaf shapes. Cotyledonary and leaf abnormalities induced by irradiated pollen are also reported in Betula nigra (Clausen, 1973a,b). Occurrence of leaves of abnormal leaf shapes in S. khasianum and S. indicum plants raised from irradiated seeds has also been reported by Ravindran (1981).

Plastochron index is a useful tool for assessing plant growth and development (Maksymowych, 1973). Irradiation greatly affects growth and development of plants raised from irradiated seeds (Gunckel and Sparrow, 1961) and irradiated pollen (Brewbaker and Emery, 1962). In the present study, however, plant height, plastochron index, leaf size, number of spines on leaves and spine frequency on leaves of S. khasianum plants raised from seeds produced due to 5 krad irradiated pollen were not affected vis-a-vis control. In a previous study

irradiation of S. khasianum seeds although did not influence plant height, plastochron index was significantly reduced by high doses (15 krad) of gamma rays (Ravindran, 1981). Thus unlike high doses of gamma rays low dose (5 krad) does not affect plant growth either in S. khasianum plants raised from irradiated seeds or in progenies of irradiated pollen.

In the present study even though the 6th leaf of plants of diverse 5 krad phenotypes did not differ from control, the leaves of the plants of diverse 5 krad phenotypes were significantly smaller than control leaves, on an average. According to Chauhan (1978b) and Ravindran (1981) in S. khasianum plants raised from irradiated seeds leaf length was more sensitive to gamma radiation than leaf breadth. However, in the present investigation leaf breadth was more sensitive than leaf length in phenotypes produced as a result of pollen irradiation. Thus seed irradiation and pollen irradiation differ in their effect on leaf expansion.

In 5 krad S. khasianum plants eventhough the number of spines on leaf in phenotypes I, III and IV did not differ from control, the spine number was greatly reduced in comparison to control in phenotype II plants. The spine frequency was, however, greatly enhanced in phenotype III and IV. Increase in spine intensity was

apparently due to reduced leaf size. Gamma irradiation of seeds also reduce the number of spines on leaves of S. khasianum and S. indicum plants (Ravindran, 1981). Ravindran (1981) observed that despite the reduction in spine number the leaves of S. khasianum were more spiny due to gamma rays induced inhibition of leaf expansion. Thus the effects of gamma radiation on spine number and spine intensity in S. khasianum plants raised from irradiated seeds or progenies of irradiated pollen are primarily similar.

In the present study spine size on both upper and lower surfaces of leaves of plants belonging to phenotype IV and only on lower leaf surface of phenotype II plants was significantly reduced. Reduction in the size of spines present on the leaves due to irradiation is reported in literature (Chauhan, 1978b; Ravindran, 1981; Sarmah, 1989). In the phenotype II plants the angle of spine was less in comparison to other phenotypes including control. Thus radiation made spines less erect which may reduce the degree of spininess of such S. khasianum plants.

The irradiated pollen caused both inhibition and stimulation of photosynthetic pigments particularly chlorophylls. However, carotenoid content increased in all the phenotypes of S. khasianum. Radiation-induced

inhibition/stimulation of chlorophylls is also reported in leaves of Vigna sinensis (Reddy et al., 1984). Reduction of chlorophyll pigments in plants raised from irradiated seeds has been attributed to the damage of leaf tissues (Woymorowska, 1972) which may be the case in present study as well. In Populus tremuloides chlorophyll mutants in F_1 progenies were recovered following pollen irradiation (Brewbaker and Emery, 1962) which suggests that photosynthetic pigments are also affected due to irradiated pollen. However, the mechanism by which irradiated pollen influences photosynthetic pigments is not well understood.

The number of flowers per plant was significantly increased in phenotype II due to an increase in the number of flowers per cluster and total number of clusters per plant. Unlike phenotype II, in phenotype III, the number of flowers per cluster was significantly reduced in comparison to control even though the total number of flowers per plant did not differ from control plants. Increased production of flowers due to ionizing radiations is reported in many plants (Gunckel and Sparrow, 1961) including tomato (Kahan, 1974) and S. khasianum (Ravindran, 1981). According to Gunckel (1965) it is tempting to think that the flowering response to irradiation might be due to auxin destruction, release of

a florigen, formation or destruction of an inhibitor or mobilization of materials. However, no satisfactory explanation has been found to date.

In the present study, radiation-induced damage was also reflected in modification of floral organs such as number of sepals and petals, abnormal development of stamen and pistil, modification of stamen into pistil-like structure and fusion of stamen and pistil. Abnormal or deformed flowers due to irradiated pollen have been reported in many plants (Brewbaker and Emery, 1962). Ravindran (1981) also reported occurrence of abnormal flowers in S. khasianum. Many morphological abnormalities including deformed flowers in immediate progenies of irradiated pollen have revealed cytological aberrations (Brewbaker and Emery, 1962). These cytological aberrants in irradiated pollen progenies primarily consist of deletion and translocation of chromosomes and aneuploids (Brewbaker and Emery, 1962). In rice plants due to gamma irradiation of pollen loss of paternal alleles at more than one locus was common and the progenies that had lost one or more paternal alleles showed some form of abnormalities such as stunted growth, abnormal panicles and deformed florets (Chin and Gordon, 1989).

In the present investigation 5 krad phenotypes of S. khasianum also showed reduced flower and anther size (phenotypes II and III). However, in phenotype IV flower

size was not affected and anther size was significantly increased. Such reduction/increase in size of flowers or floral parts are clear indication of radiation-induced damages in progenies of irradiated seeds (Gunckel and Sparrow, 1961) and irradiated pollen (Brewbaker and Emery, 1962).

In all the phenotypes, excepting phenotype I, pollen stainability was greatly reduced. This could be because in plants of these phenotypes (II-IV) only insignificant fertile pollen developed and most of the pollen formed in the anther aborted during development. The sterility of pollen in these phenotypes (II-IV) was further supported by hand pollinating stigma with the pollen of these phenotypes (II-IV). Pollination of stigma with the pollen of phenotypes (II-IV) failed to induce any fruiting while pollination with normal pollen set fruit. Thus while pollen from phenotypes II-IV were sterile the pistil in these phenotypes like control was fertile. Further, all these phenotypes (II-IV) in natural condition developed fruits. Therefore, it may be concluded that S. khasianum plants of phenotypes (II-IV), produced as a result of 5 krad irradiated pollen, were female fertile and male sterile. Pollen sterility as an index of radiation genetic damage was suggested by Brewbaker and Emery (1962). Translocations involving loss of genes may be the

reason for sterility in plants from irradiated pollen (Brewbaker and Emery, 1962). Pollen sterility finds its utility in producing hybrid vigor in plant breeding (Gabelman, 1956; Duvick, 1966; Zhang, 1990). Male sterility is necessary to prevent the maternal lines (seed line) from sibbing where hand emasculation is not feasible (Van Marrewijk et al., 1986).

In the present study except in phenotype I, occurrence of long-styled flowers was greatly improved in all other phenotypes of 5 krad plant of S. khasianum. Interestingly, in one of the phenotypes (Phenotype IV) all the flowers produced on the plant were long-styled flowers and 3-6 (mean 4.33) long-styled flowers were produced in a cluster. Enhanced production of long-styled flowers due to low dose (5 krad) gamma irradiation of S. khasianum seeds has been reported earlier (Chauhan and Ravindran, 1979; Ravindran, 1981). Growth hormones play an important role in sex expression in plants (Heslop-Harrison, 1957; Chailakhyan, 1979). Many workers have suggested possible involvement of growth hormones in regulation of stylar heteromorphism in solanums (Wakhloo, 1976; Baksh et al., 1979; Chauhan and Ravindran, 1980; Ravindran, 1981; Solomon, 1985; Joshee, 1986; Chauhan and Joshee, 1987). Chauhan and Joshee (1987) suggested that a balance of kinetin and GA_3 could be involved in the production of long- and short-styled flowers in S. khasianum. Chauhan

and Ravindran (1980) had also stated that radiation-induced inhibition of long-styled flower production in S. khasianum could be overcome by spraying kinetin. Thus phenotype IV may be richer in kinetin in comparison to other phenotypes and control. Radiation induced cytokinin synthesis in tissues is reported in literature (Pandey et al., 1978).

At maturity two phenotypes (Phenotypes II and III) were much shorter than control. Such reduced height or arrested growth is a common morphological change induced as a result of gamma radiation and has been reported in many plant species (Gunckel and Sparrow, 1961; Brewbaker and Emery, 1962). Although plant height was reduced in 5 krad plants of S. khasianum lateral growth was stimulated. Stimulation of lateral growth in these phenotypes were reflected in increased number of branches. Ionizing radiations are known to break apical dominance and accelerate lateral branches (Sax, 1963). According to Skoog (1935) radiation reduces or inactivates the auxin production and promotes lateral bud development by releasing their arrest. Radiation-induced stimulation of side shoots is reported in literature (Kahan, 1973; Shamsi and Bajwa, 1978; Ravindran, 1981).

The number of berries produced per plant in 5 krad plants of S. khasianum differed from one phenotype to another. In Phenotype IV the number of berries was enhanced, but in Phenotype III the number was reduced compared to control while in other phenotypes it did not differ from control. Increased berry number per plant is

reflected in increased production of long-styled flowers in phenotype IV. However, in phenotype III though the frequency of long-styled flowers was higher than control, number of berries per plant was significantly reduced. This could be due to the early abscission of flowers due to radiation. Such early abscission of flower induced due to ionizing radiation is reported in literature (Gunckel and Sparrow, 1961; Brewbaker and Emery, 1962).

Number of berry per cluster, in all the phenotypes in the present study, did not differ from control.

But for phenotype I berry size, berrry volume and berry weight was greatly reduced in all the phenotypes of S. khasianum. Further, compared to control berries, the berries of these phenotypes had higher moisture content. Inhibition of S. khasianum berry size and weight due to gamma radiation was also observed by Ravindran (1981).

In the present study number of seeds produced per berry in different phenotypes of 5 krad plants was also greatly impaired; seed weight, however, did not differ from control seeds. Reduced number of seeds due to gamma radiation has also been reported in S. indicum (Ravindran, 1981). The reduction in the number of seeds due to radiation could be because of disturbances in the post-fertilization development of seeds.

Table 29. Germination percentage of control and seeds formed by irradiated (5 krad) pollen in S. khasianum

Days	In vitro (25°C)		In pots Temp. Max.=20°C. Min.=10°C	
	Control (%)	5 krad (%)	Control (%)	5 krad. (%)
20	17	5	0	0
30	77	18	0	0
40	95	30	0	0
50	95	39	36.67±2.96	11.67±0.88*** (-68.18)
60	95	43	89.33±2.18	28.00±0.58*** (-68.66)
70	95	43	91.67±2.40	33.00±1.15*** (-64.00)
80	95	43	93.33±2.03	37.67±2.40*** (-59.64)
90	95	43	93.33±2.03	43.00±3.51*** (-53.93)

± SE

*** Significantly different from control at p = 0.001

Figures in parentheses indicate percent inhibition (-) over control

Table 30. **Number of abnormal seedlings developed from seeds produced by irradiated (5 krad) pollen in *S. khasianum***

Treatment	Number of abnormal seedlings	Number of normal seedlings	Total seedlings examined
Control	0	280	280
5 krad	10	119	129

Table 31. Morphological characters of plants obtained from control and seeds formed by irradiated (5 krad) pollen in S. khasianum

Phenotype	6th week old plants		6th leaf at maturity			Number of spines on the 6th leaf			Spine frequency
	Height (cm)	Plastochron index	Length (cm)	Breadth (cm)	Area (cm^2)	Upper surface	Lower surface	Total	
Control	10.00±0.40	8.60±0.25	13.08±0.49	16.79±0.75	219.61±16.69	17.56±1.23	21.44±1.95	39.00±2.85	0.18±0.01
I	9.36±0.55	8.59±0.23	13.01±0.06	17.40±0.58	227.94±8.21	15.50±0.50	19.83±1.22	35.33±1.33	0.16±0.01
II	8.50	9.50	12.50	15.80	197.50	18.00	17.00	35.00	0.18
III	9.50	8.50	5.70*** (-56.42)	6.90** (-58.90)	39.33** (-82.09)	15.00	16.00	31.00	0.79*** (+338.89)
IV	10.00	9.00	13.10	17.80	229.25	17.00	19.00	36.00	0.16

± SE

** Significantly different from control at p = 0.01

*** Significantly different from control at p = 0.001

Figures in parentheses indicate percent stimulation(+)/inhibition(-) over control

Phenotypes :

I Dicotyledon Normal leaf type

II Dicotyledon Triangular leaf type

III Dicotyledon Roundish leaf type

IV Tricotyledon all fused together at the base.

Table 32. Leaf and spine characters in control and 5 krad plants of S. khasianum

Phenotype	Average leaf size			Average number of spines on leaf	Spine frequency	Length of spine (cm)		Angle of spine (°)
	Length (cm)	Breadth (cm)	Area (cm^2)			Upper surface of leaf	Lower surface of leaf	
Control	18.38±0.24	22.70±0.68	417.23±16.87	47.00±2.60	0.11±0.009	1.65±0.06	1.33±0.05	75.25±3.42
I	18.13±0.59	23.13±1.48	419.35±39.26	44.00±2.97	0.10±0.005	1.58±0.09	1.33±0.09	76.25±2.93
II	11.50±0.50*** (-37.43)	11.00±0.61*** (-51.54)	126.50±13.09*** (-69.68)	16.00±1.87*** (-65.60)	0.13±0.010	1.60±0.18	1.08±0.09* (-18.80)	60.75±10.59* (-19.26)
III	10.75±0.48*** (-41.51)	11.75±0.66*** (-48.24)	126.31±11.75*** (-69.73)	52.00±5.23	0.41±0.024*** (+272.73)	1.73±0.08	1.25±0.06	81.25±1.75
IV	15.88±0.55*** (-24.48)	15.75±0.43*** (-30.62)	218.61±14.70*** (-47.60)	47.00±2.02	0.21±0.024*** (+90.91)	0.81±0.20*** (-51.52)	0.90±0.11*** (-32.33)	71.25±4.27

± SE

* Significantly different from control at p = 0.05

*** Significantly different from control at p = 0.001

Values in parentheses indicate percent stimulation(+)/inhibition(-) over control

Phenotypes :

I Dicotyledon Normal leaf type

II Dicotyledon Triangular leaf type

III Dicotyledon Roundish leaf type

IV Tricotyledon all fused together at the base.

Table 33. Pigment variation in plants obtained from control and seeds formed from irradiated (5 krad) pollen in S. khasianum

Phenotype	Photosynthetic pigments (mg/g)			
	Chlorophyll 'a'	Chlorophyll 'b'	Total chlorophyll	Carotenoids
Control	1.68±0.06	1.66±0.10	3.34±0.16	1.53±0.95
I	1.39±0.03* (-17.26)	1.33±0.08** (-19.88)	2.70±0.11** (-19.16)	1.57±0.23* (+2.61)
II	2.20±0.08*** (+30.95)	2.06±0.02*** (+24.10)	4.22±0.10*** (+26.35)	2.50±0.41*** (+63.40)
III	1.93±0.10* (+14.88)	1.79±0.06	3.71±0.16	1.64±0.70*** (+7.19)

± SE

* Significantly different from control at p = 0.05

** Significantly different from control at p = 0.01

*** Significantly different from control at p = 0.001

Values in parentheses indicate percent stimulation (+)/ inhibition (-) over control.

Phenotypes :

I Dicotyledon Normal leaf type

II Dicotyledon Triangular leaf type

III Dicotyledon Roundish leaf type

Table 34. Production of flowers and long-styled frequency in various 5 krad plants of S. khasianum

Phenotype	No. of flower cluster/plant	Total flowers/plant	No. of flowers/cluster		Total long-styled flowers/plant	No. of long-styled flowers/cluster		Long-styled flower frequency (%)
			Range	Mean		Range	Mean	
Control	15.00±0.50	53.00±2.68	1-6	3.64±0.13	18.67±2.49	1-4	1.28±0.83	35.22±2.79
I	14.00±0.81	51.00±2.33	1-6	3.49±0.21	20.57±2.06	1-4	1.45±0.13	41.26±2.12
II	61.00*** (+306.67)	354.00*** (+567.92)	3-11	5.74±0.26*** (+57.69)	290.00*** (+1453.29)	2-11	4.75±0.26*** (+271.09)	82.75±2.12** (+134.95)
III	23.00±1.50	59.00±25.50	1-6	2.60±0.44* (-28.57)	57.00±24.00*** (+205.30)	1-5	2.53±0.50*** (+97.66)	97.44±4.64*** (+176.66)
IV	12.00	64.00	3-6	4.33±0.21	64.00*** (+242.80)	3-6	4.33±0.21*** (+238.28)	100.00±0.00*** (+183.93)

± SE

* Significantly different from control at p = 0.05

** Significantly different from control at p = 0.01

*** Significantly different from control at p = 0.001

Figures in parentheses indicate percent stimulation(+)/inhibition(-) over control

Phenotypes :

I Dicotyledon Normal leaf type

II Dictotyledon Triangular leaf type

III Dicotyledon Roundish leaf type

IV Tricotyledon all fused together at the base.

Table 35. Variations in floral morphology and pollen stainability of control and 5 krad plants of S. khasianum

Phenotype	Flower length at anthesis (mm)	No. of sepals	No. of petals	No. of stamens	Long-styled flower		Short-styled flower	
					Anther length (mm)	Pollen stainability (%)	Anther length (mm)	Pollen stainability (%)
Control	10.60±0.19	5	5	5	6.93±0.05	95.02±1.47	6.89±0.03	93.78±1.51
I	10.35±0.21	5	5	5	6.92±0.02	92.60±1.68	6.88±0.03	91.50±1.44
II	9.50±0.31** (-10.38)	4-6	4-6	4-7	4.29±0.08*** (-38.10)	3.82±0.92*** (-95.98)	4.24±0.05*** (-38.46)	3.12±0.44*** (-96.67)
III	7.30±0.37*** (-31.13)	5	4-5	3-5	4.74±0.06*** (-31.60)	4.81±0.91*** (-94.94)	4.71±0.05*** (-31.64)	4.53±1.91*** (-95.17)
IV	10.55±0.28	5	5	5-6	7.18±0.12* (+3.61)	12.54±1.81 (-86.80)	-	-

± SE

* Significantly different from control at p = 0.05

** Significantly different from control at p = 0.01

*** Significantly different from control at p = 0.001

Figures in parentheses indicate percent stimulation(+)/inhibition(-) over control

Phenotypes :

I Dicotyledon Normal leaf type

II Dicotyledon Triangular leaf type

III Dicotyledon Roundish leaf type

IV Tricotyledon all fused together at the base.

Table 36. Berry forming ability of long-styled flowers of control and 5 krad plants of S. khasianum

Phenotype	Fruit formation			Fertility	
	Natural	Self	With normal pollen	Pistil	Pollen
Control	+	+	+	+	+
I	+	+	+	+	+
II	+	-	+	+	-
III	+	-	+	+	-
IV	+	-	+	+	-

\+ Positive response (Fertile)

\- Negative response (Sterile)

Phenotypes :

I Dicotyledon Normal leaf type

II Dicotyledon Triangular leaf type

III Dicotyledon Roundish leaf type

IV Tricotyledon all fused together at the base

Table 37. Plant growth and development and berry production in plants obtained from control and seeds produced by irradiated (5 krad) pollen of S. khasianum

Phenotype	At maturity					
	Plant height (cm)	Spread (cm)	Number of branches	Total berry/plant	No. of berry/cluster	
					Range	Mean
Control	78.00±3.43	37.83±1.69	3.44±0.24	7.78±0.49	1-3	1.28±0.08
I	77.29±2.22	29.93±2.03	4.14±0.26	7.86±0.55	1-3	1.25±0.08
II	52.00* (-33.33)	55.50* (+46.71)	15.00*** (+336.05)	10.00	1-2	1.67
III	58.00±10.00* (-25.64)	58.50±9.50** (+54.64)	9.00±0.00*** (+161.63)	4.00±1.00** (-48.59)	1-2	1.14±0.13
IV	100.00	64.00*** (+69.18)	8.00*** (+132.56)	15.00** (+92.80)	1-3	1.88

± SE

* Significantly different from control at p = 0.05

** Significatnly different from control at p = 0.01

*** Significantly different from control at p = 0.001

Figures in parentheses indicate percent stimulation(+)/inhibition(-) over control

Phenotypes :

I Dicotyledon Normal leaf type

II Dicotyledon Triangular leaf type

III Dicotyledon Roundish leaf type

IV Tricotyledon all fused together at the base.

Table 38. Characteristics of berries produced on plants from seeds produced by control and irradiated (5 krad) pollen of S. khasianum

Phenotype	Berry diameter (cm)	Berry volume (cm^3)	Berry fresh weight (g)	Berry dry weight (g)	Moisture content (%)	No. of seeds/berry	Weight of 100 seeds (mg)
Control	2.66±0.07	10.05±0.74	7.07±0.26	1.52±0.05	78.50±0.18	390.00±27.19	270.46±16.45
I	2.59±0.07	9.27±0.72	6.72±0.21	1.41±0.08	79.02±0.16	383.40±18.69	294.81±20.45
II	1.50±0.02*** (-43.61)	1.80±0.09*** (-82.09)	1.22±0.12*** (-82.74)	0.14±0.02*** (-90.79)	88.52±0.40*** (+12.76)	26.00± 3.12*** (-93.33)	306.26±30.43
III	1.53±0.10*** (-42.48)	1.91±0.35*** (-81.00)	1.42±0.28*** (-79.92)	0.22±0.05*** (-85.53)	84.51±0.87*** (+7.66)	35.00±12.04*** (-91.03)	270.04±23.16
IV	2.10±0.02*** (-21.05)	4.94±0.15*** (-50.85)	2.79±0.22*** (-60.54)	0.45±0.04*** (-70.39)	83.87±0.34 (+6.84)	62.20± 6.27*** (-84.05)	288.00±18.43

± SE

*** Significantly different from control at p = 0.001

Figures in parentheses indicate percent stimulation(+)/inhibition(-) over control

Phenotypes :

I Dicotyledon Normal leaf type

II Dicotyledon Triangular leaf type

III Dicotyledon Roundish leaf type

IV Tricotyledon all fused together at the base.

Fig. 30 : Seedling abnormalities in germinants of seeds formed due to 5 krad irradiated pollen of *S. khasianum*.

a - control seedling with 2 cotyledons

b - 5 krad seedling with 3 cotyledons all fused together at the base

c - control seedling at 4-leaf stage

d - 5 krad Triangular leaf type

e - 5 krad Roundish leaf type.

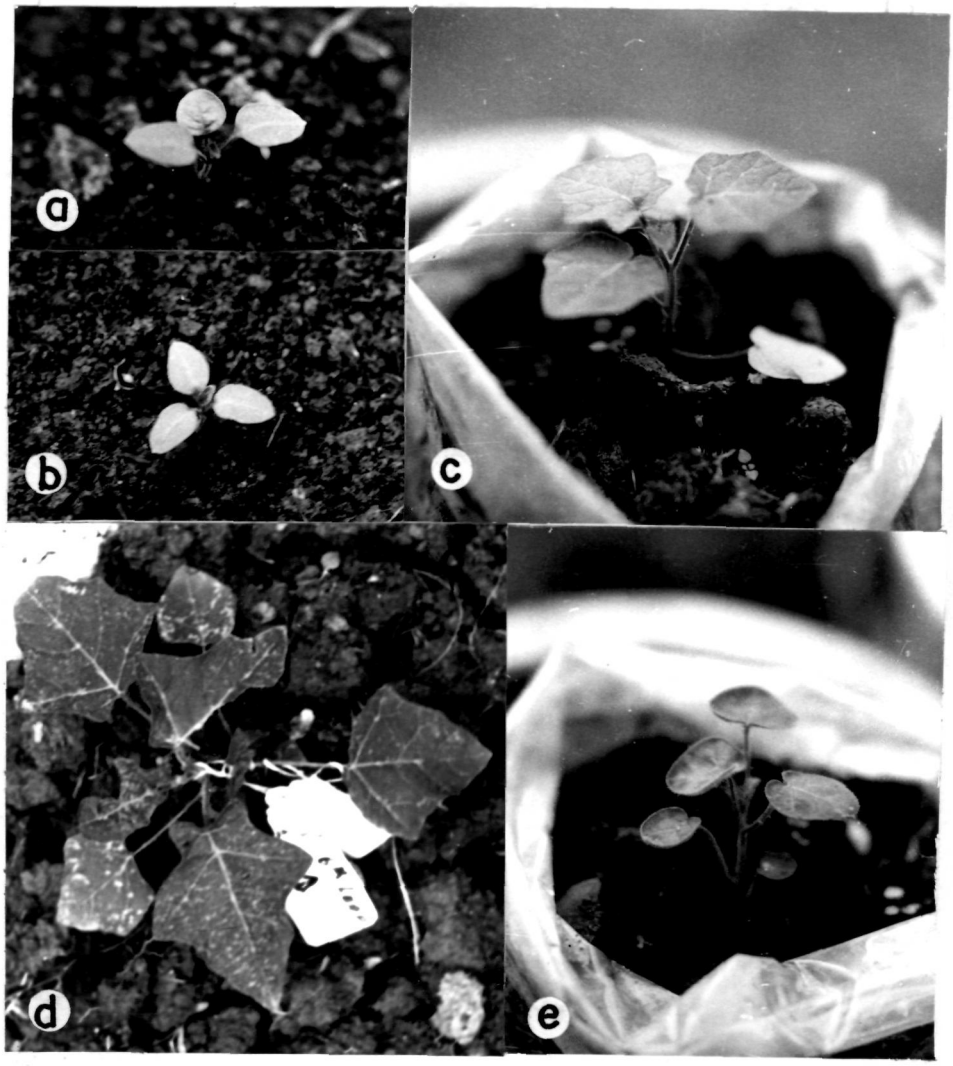


Fig.30

Fig. 31 : Leaf phenotypes in 5 krad plants of *S. khasianum*.

Cont. - Control leaf
a - 5 krad Normal leaf type
b - 5 krad Triangular leaf type
c - 5 krad Roundish leaf type

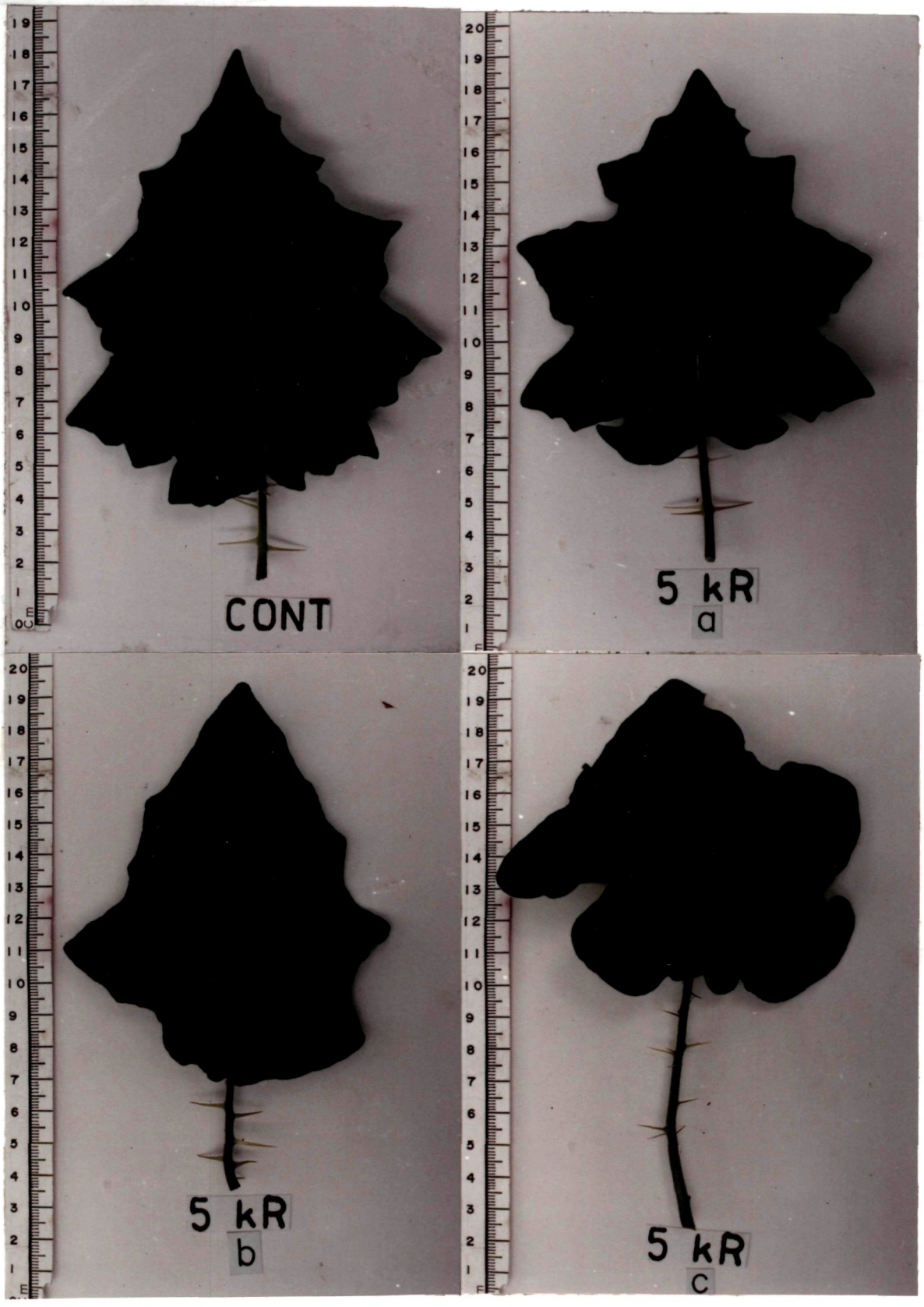


Fig. 31

Fig. 32 : 4 months old control plant and 5 krad phenotypes of *S. khasianum*.

a - control plant

b - 5 krad normal leaf type (Phenotype I)

c - 5 krad Triangular leaf type (Phenotype II)

d - 5 krad Roundish leaf type (Phenotype III)

e - 5 krad Tricotyledon all fused together at the base (Phenotype IV).

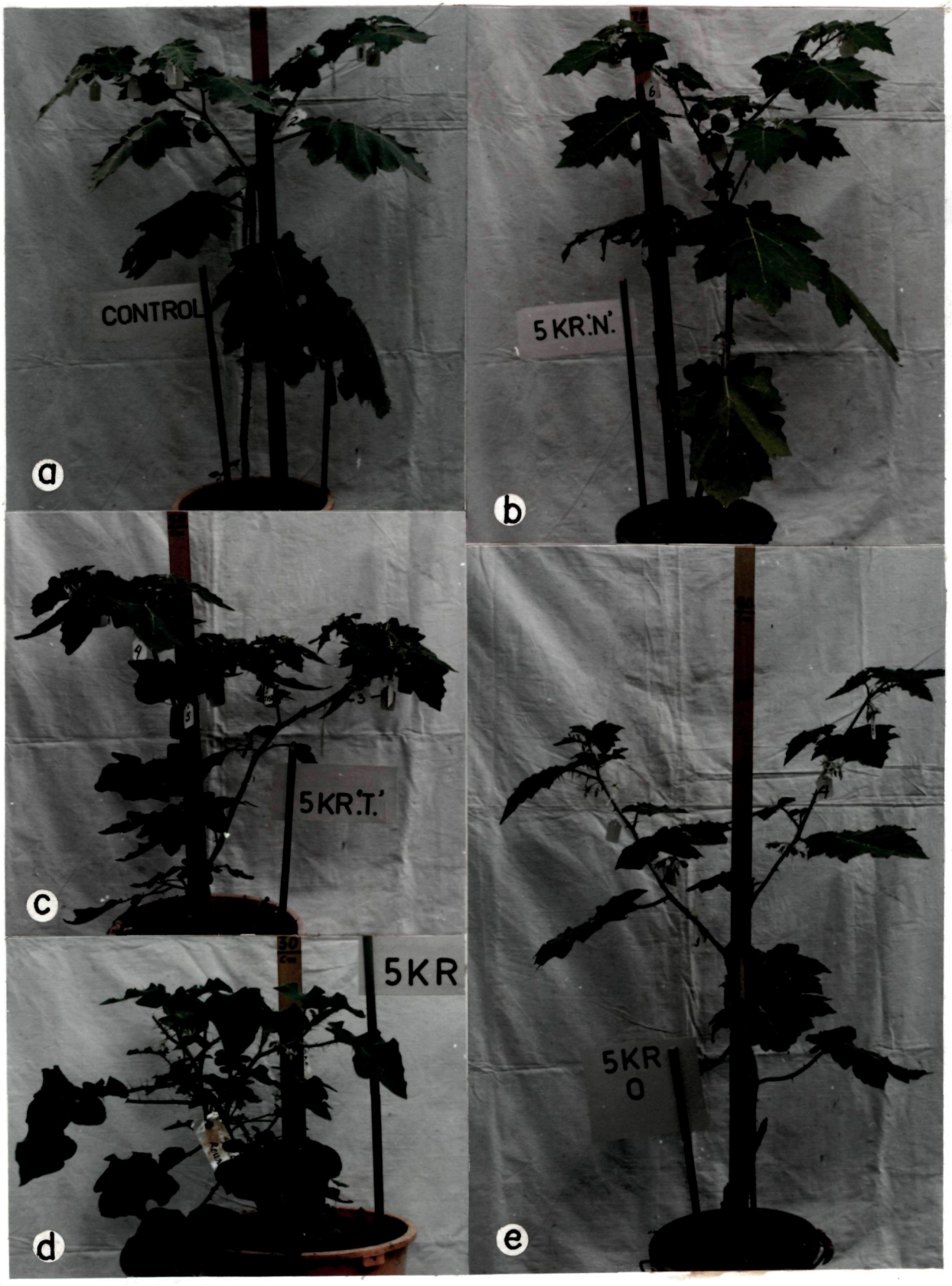


Fig. 32

Fig. 33 : Flower abnormalities in 5 krad phenotypes of *S. khasianum*.

a - control flower

b - 5 krad normal flower

c - 5 krad flower with abnormal stamen (Arrow mark)

d - 5 krad flower with broad stigma (Arrow mark)

e - 5 krad flower with splitted stigma (Arrow mark)

f - 5 krad flower with modified stamen into pistil like structure (Arrow mark)

g - 5 krad flower with stamen fused together with pistil (Arrow mark).

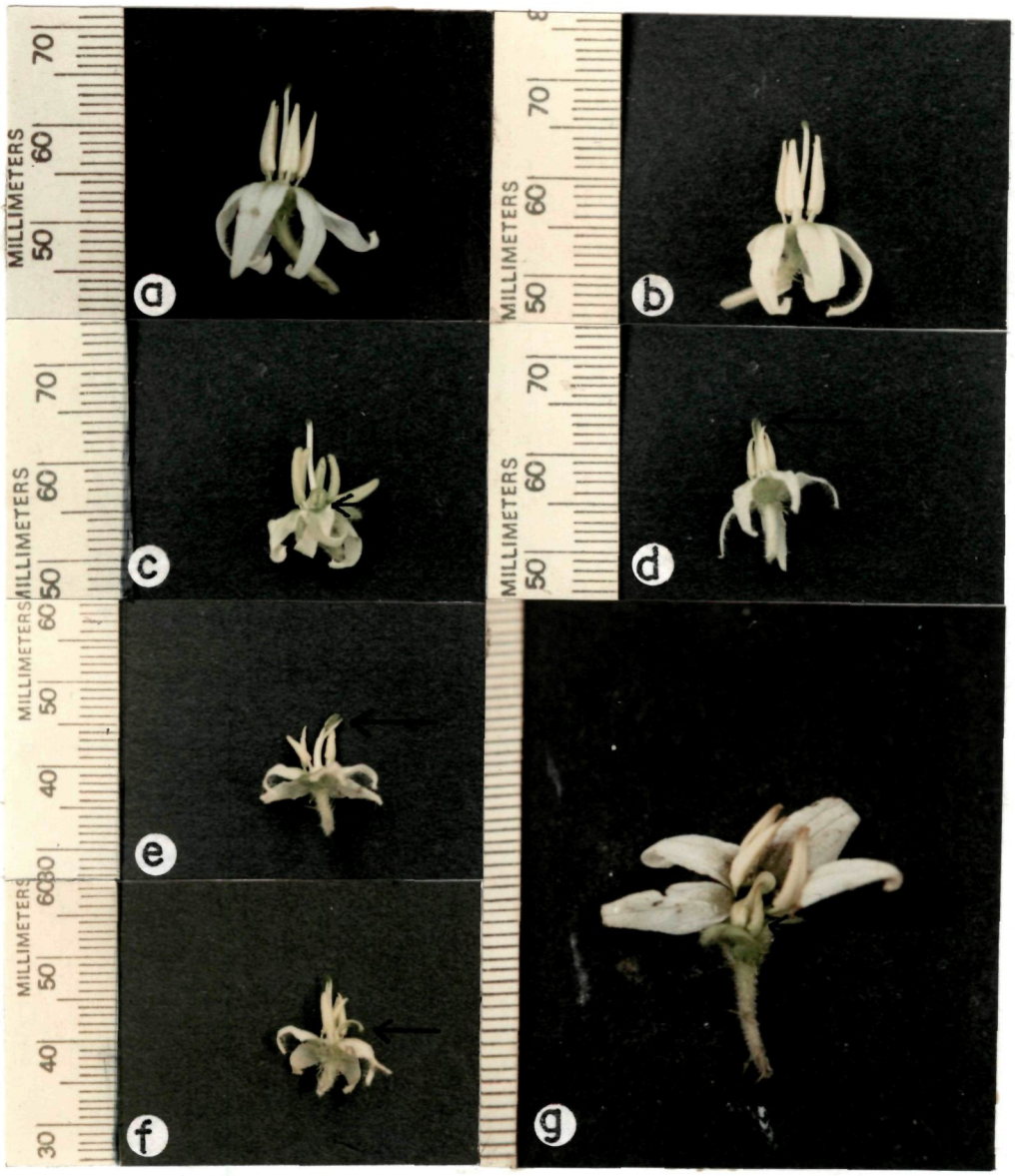


Fig.33

Fig. 34 : Pollen grains from control and 5 krad phenotypes of S. khasianum.

a - Pollen from control plant
b - Pollen from 5 krad Phenotype I
c - Pollen from 5 krad Phenotype II
d - Pollen from 5 krad Phenotype III
e - Pollen from 5 krad Phenotype IV

Red stained pollen - Normal viable pollen
Unstained pollen - Aborted non-viable pollen

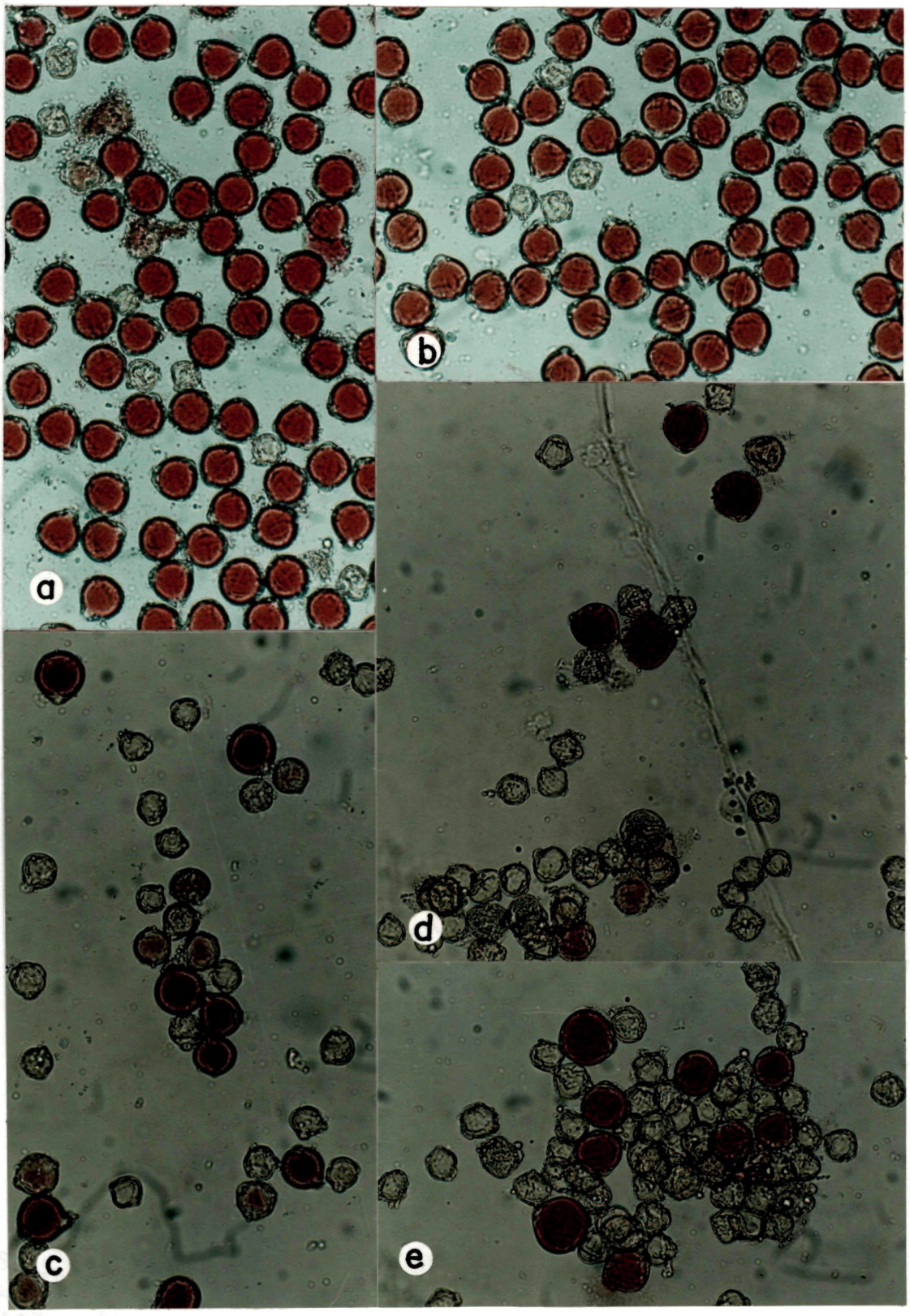


Fig.34

Fig. 35 : Berries of control and 5 krad phenotypes formed from open pollination in S. khasianum.

a - control
b - 5 krad Phenotype I
c - 5 krad Phenotype II
d - 5 krad Phenotype III
e - 5 krad Phenotype IV

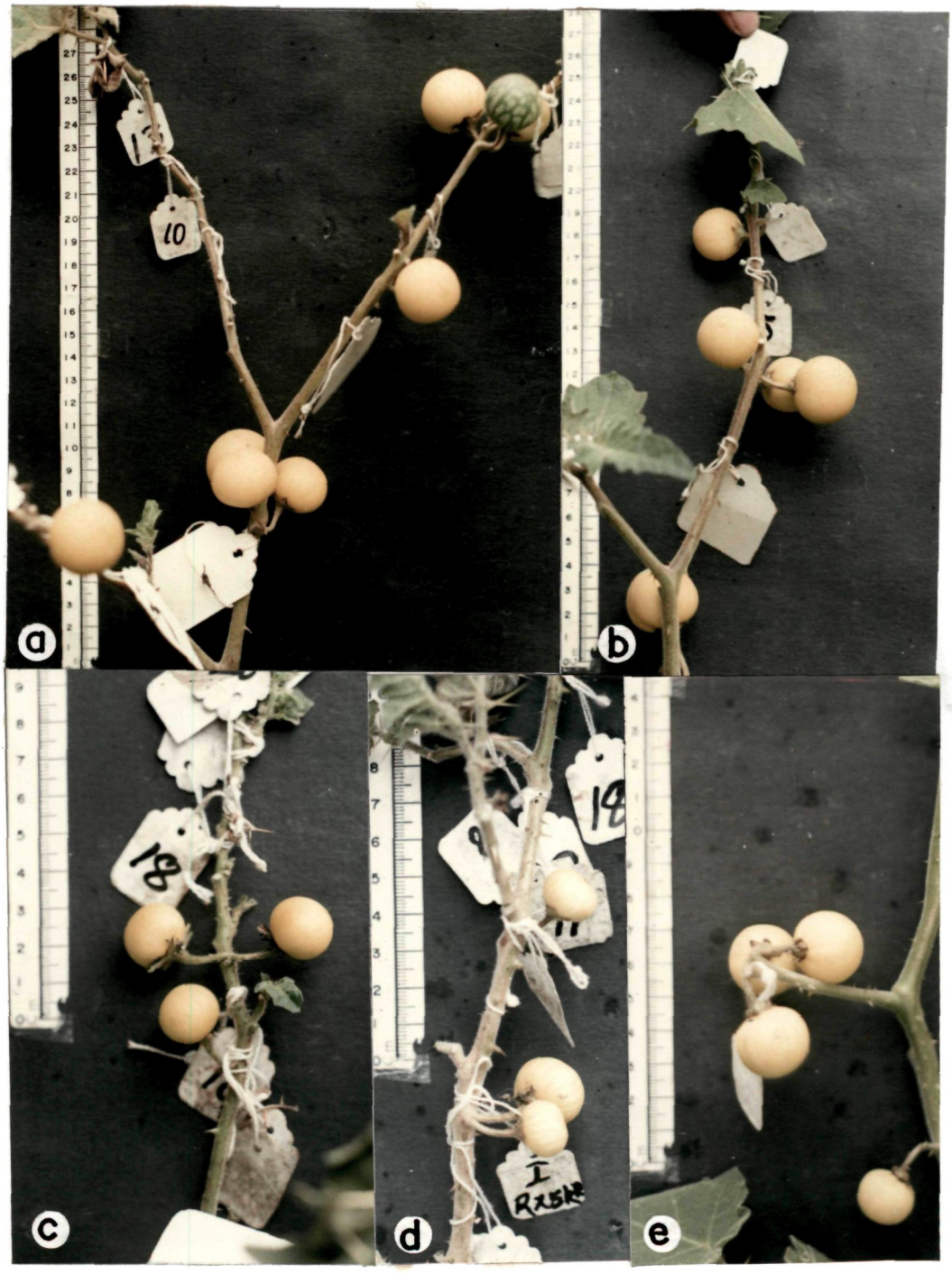


Fig.35

Fig. 36 : Showing size of berries formed on control and various 5 krad phenotypes of *S. khasianum*.

1,2 - control
3,4 - 5 krad Phenotype I
5,6 - 5 krad Phenotype IV
7 - 5 krad Phenotype II
8 - 5 krad Phenotype III

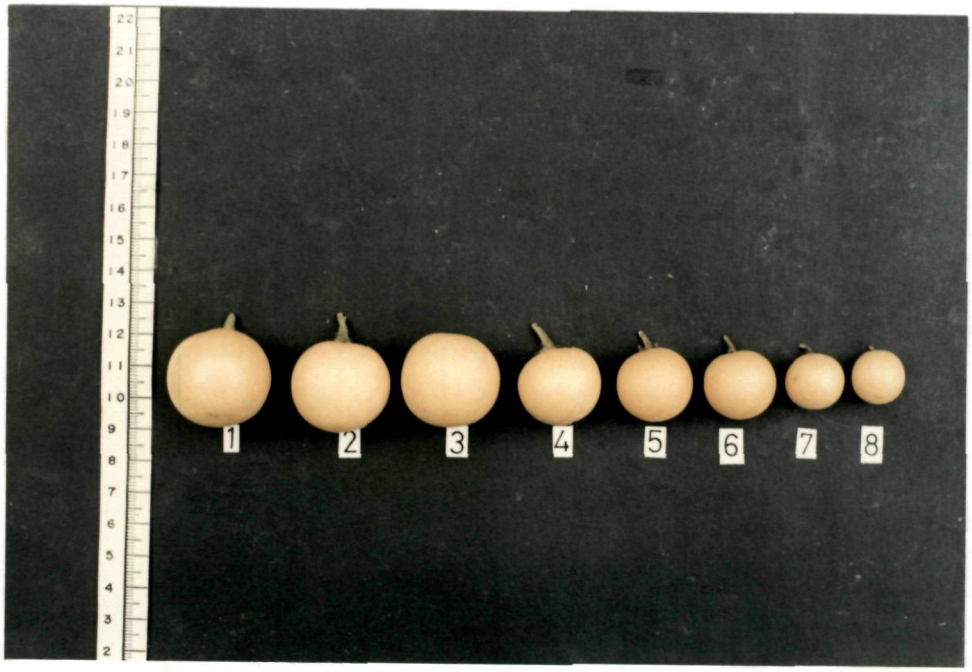


Fig.36

Chapter-VI

FLOWER BUD DEVELOPMENT OF S. KHASIANUM IN VITRO

INTRODUCTION

Similar to many other solanums, Solanum khasianum exhibits stylar heteromorphism (Murty and Abraham, 1975). As a result two types of flowers, long-styled and short-styled flowers are produced in the same inflorescence. Long-styled flowers are female fertile and bear fruits while short-styled flowers are female sterile and bear no fruit. The frequency of long-styled flowers in this species ranges between 30-40% only (Kaul and Zutshi, 1977). Hence berry yield is greatly affected. Various factors have been attributed to the ocurrence of stylar heteromorphism in solanums. Environmental conditions such

as day length (Pal and Singh, 1943) and sunlight intensity (Solomon, 1985) are suggested to have some influence on sex expression in solanums. Hossain (1973) suggested that it is due to an exhaustion or inadequate supply of some substance responsible for pistil growth right from the bud stage. Similar cnclusions were drawn by Martin (1972) and Reddy and Bahadur (1977). Nutrition and water stress influence sex expression in S. carolinense (Solomon, 1985). Potassium content in the leaves have some bearing on the production of long- and short-styled flowers in S. sisymbrifolium; plants with high potassium level produce less short-styled flowers (Wakhloo, 1975). Heslop-Harrison (1957) postulated that auxin regulates sex expression in plants, low level of auxin favours the development of stamens while high level favours pistil development. Chailakhyan (1979) also suggested the involvement of GA_3 (maleness) and cytokinin (femaleness) in regulation of sex expression in plants. However, Baksh et al. (1979) speculated the involvement of two separate auxins for stamen and pistil development in Solanum species. Chauhan and Ravindran (1980) suggested the involvement of kinetin (long-styles) and GA_3 (short-styles) in the expression of stylar heteromorphism in S. khasianum. In combination treatments of kinetin and GA_3 increasing concentrations of kinetin/GA_3 determined the ratio of long-/short-styled flowers development (Joshee, 1986; Chauhan and Joshee, 1987). Thus a balance of

kinetin and GA_3 could be involved in the production of long- and short-styled flowers in S. khasianum (Chauhan and Joshee, 1987). Wakhloo (1976) also suggested potassium mediated GA_3 regulation to be responsible for female sterile flowers in S. sisymbrifolium. Increase or decrease of long-/short-styled flowers by spraying growth hormones on the plant (ABA, BAP - long-styled flowers, IAA, GA_3-short-styled flowers) was also observed in S. carolinense by Solomon (1985).

The technique of culturing young excised flower buds affords a potentially useful tool for study concerned with the control of flower morphogenesis; influences of growth regulators and nutrients without the presence of intervening vegetative tissue (Konar and Kitchlue, 1982). In several plant species flower buds have been successfully cultured through to anthesis using different growth hormones/regulators of various concentrations (Galun et al., 1962, 1963; Tepfer et al., 1963, 1966; Blake, 1966, 1969; Hicks and Sussex, 1970; Bilderback, 1971, 1972; Rastogi and Sawhney, 1986, 1988). Such attempt to grow excised flower buds in vitro, however, has not been made in S. khasianum.

The potentiality of the flower buds for growth and development in vitro varies from species to species and appropriate culture conditions and nutrients are essential

for normal development *in vitro* (Konar and Kitchlue, 1982). Therefore, in the present study a preliminary attempt has been made to culture excised flower buds of *S. khasianum* in a selected medium supplemented with few growth hormones to study the response of the flower buds *in vitro*.

MATERIAL AND METHOD

Medium Preparation

For *in vitro* culturing of excised flower buds of *S. khasianum*, the widely acclaimed MS basal medium (Murashige and Skoog, 1962) was used. The composition of the basal medium is as follows :

Ingredient	mg/l
A. Macronutrients	
$(NH_4)NO_3$	1650
KNO_3	1900
$CaCl_2.2H_2O$	440
$MgSO_4.6H_2O$	370
KH_2PO_4	170
B. Micronutrients	
$MnSO_4.4H_2O$	22.3
$ZNSO_4.4H_2O$	8.6
H_3BO_3	6.2

KI	0.83
$NaMoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025

C. Iron

$FeSO_4.7H_2O$	27.8
Na_2EDTA	33.6

D. Vitamins

Nicotinic acid	0.5
Pyridoxine HCl	0.5
Thiamine HCl	0.1
Glycine	2.0
Myo-inositol	100
Sucrose	30,000
Agar	1% (W/V)

The pH of the medium was adjusted to 5.7 with 1N NaOH/ 1N HCl before adding agar. Subsequent to addition of agar in the medium, the medium was gently warmed so that agar melted and dissolved. Various concentrations of growth hormones (kinetin, BAP, IAA and NAA)* were added separately so as to make the final concentrations

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* For preparing [redacted] stock solutions of growth hormones kinetin and BAP were first dissolved in a few drops of IN HCl or ethanol while IAA and NAA were dissolved in a few drops of 1N NaOH solution. After dissolving the growth hormone solution volume was made up using double glass distilled water.

10^{-12}- 10^{-4}M. About 25-30 ml of the medium was poured into 100 ml conical flask. The medium was prepared using double glass distilled water. For each concentration of growth hormones 5 flasks were maintained. Appropriate controls were maintained, in which no growth hormone was added. The flasks were plugged with cotton plugs and capped with aluminium foil. These flasks were then autoclaved at 15 lb/in^2 pressure and 121°C for 15 minutes. Subsequent to autoclaving the flasks were kept in aseptic conditions pending inoculation with flower buds.

Preparation of Flower Buds

Tender shoots of S. khasianum bearing inflorescence having 1-2 flower buds measuring 2.5-3 mm were cut from plants grown in the experimental field. The shoots were trimmed carefully by removing excess leaves and washed several times in tap water. The shoots were then surface sterilized by completely submerging the shoots for 15 minutes in 5-7% (v/v) sodium hypochlorite solution prepared in double glass distilled water and having a few drops of a wetting agent Tween 20. The flask was shaken periodically to allow complete wetting of the shoots. These were then washed 3-4 times with sterilized double glass distilled water. Again the excised inflorescences were placed in 1% (w/v) mercuric chloride solution for 1-2 minutes and agitated to ensure complete surface

sterilization. The shoots were then transferred to another flask and washed several times with sterilized double glass distilled water to ensure complete removal of mercuric chloride solution. For the purpose of surface sterilization and washings of excised inflorescences only sterilized glasswares were used. The shoots were then taken out and the flower buds were carefully cut to size, leaving a small portion of the stem attached to the inflorescence, with the help of a sterilized blade/microsurgery knife and forceps in a sterilized petriplate. The flower buds were then placed on a sterilized 1% agar-plate in a petridish for easy/quick handling during inoculation. All these processes of surface sterilization, washing and cutting of the plant material and inoculation were done under aseptic condition using a laminar air-flow chamber also fitted with ultraviolet light.

Inoculation

During inoculation one flower bud was transferred onto the medium in each flask with the help of a sterilized inoculating needle. The stem portion of the flower buds was slightly inserted into the medium. As soon as inoculation was over each flask was re-plugged with the cotton plug and capped with aluminium foil.

Incubation

The inoculated flasks were incubated on culture racks fitted with light (3 white cool fluorescence tube light - 40 watts, 120 cm long and 2 incandescent bulbs - 25 watts for each shelf) giving 3,500 lux light intensity. The flasks were exposed to 16 hours light and 8 hours dark condition. During this period the temperature was maintained at 25°-28°C with the help of hot air blower. At the end of 15-20 days of culture the flowers/buds were taken out for assessment. Each experiment was repeated at least three times.

The cultured flowers were fixed in FAA (5 ml formaldehyde + 5 ml glacial acetic acid + 90 ml 50% ethanol). These flowers were dehydrated through TBA series and embedded in praffin. Sections (15 µm) were cut and stained as described in Chapter IV.

RESULTS

Table 39 presents data on the influence of varying bud sizes (1.32, 2.39 and 3.23 mm) on bud growth and pistil elongation in S. khasianum buds cultured on MS medium (Murashige and Skoog, 1962) for 15 days. Considerable growth of floral bud and pistil occurred in cultured flower buds of all the three sizes. However, maximum floral bud growth was evident in bud explants of 1.32 mm. In these explants the increase in size of bud

was 309.09% (**Table 39**). The increase in size of flower buds of 2.39 mm and 3.23 mm during culturing was 188.70% and 157.89% respectively. During culturing maximum elongation of the pistil was evident in floral bud explants of 3.23 mm. Minimum pistil elongation occurred in bud explants of 1.32 mm (**Table 39**). The growth of pistil was intermediate in the floral bud explants measuring 2.39 mm (**Table 39**). Therefore for subsequent experimentation floral buds measuring 3.23 mm were used.

Tables 40, 41, 42 and 43 give data on the effect of various concentrations of benzylaminopurine (BAP), kinetin (KN), Indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA) on *in vitro* development of excised floral buds of *S. khasianum*. Floral buds grown on medium devoid of growth hormones (control) developed abnormal flower (**Fig. 37.1A,a**) (only 6.67% of the cultured control floral buds could develop into flowers) (**Table 40**). A comparison of *S. khasianum* flowers, developed *in vivo* and *in vitro* on medium devoid of growth hormones, at anthesis revealed that the flowers and floral parts developed *in vitro* were smaller in size (**Table 40**).

The ovules in long-styled flower at anthesis reveal an organised Polygonum type of embryo sac (**Fig. 38.1a**). The embryo sac is bounded by a layer of endothelium. The style is distinguishable into an outermost epidermal

layer followed by layers of parenchymatous cortex and centrally differentiated small, narrow and elongated cells the transmission tissue (Fig. 38.2a). The stigma is bilobed and composed of an epidermis followed by parenchymatous stigmatic tissue having intercellular spaces. Each lobe of the stigma receives a vascular strand. Major part of the stigma is made up of transmission tissue. Stigmatic papillae are present all along the surface of stigma (Fig. 38.2a). Development of famele gametophyte, in pistil and anther of floral buds cultured on medium devoid of growth hormones (control) was arrested and no nuclei could be found in the embryo sac (Figs.38.1b, 38.2b & 38.3b). In comparison to control flower buds, incorporation of BAP at various concentrations (10^{-12}- 10^{-4}M) improved in vitro development of excised flower buds (Table 40; Figs. 37.2A,a-E,e). Percentage of flower buds developing into near normal flower also improved and 25% floral buds developed into flowers on medium supplemented with 10^{-10}M of BAP (Table 40). However, high or low concentrations of BAP reduced number of floral buds developing into flowers (Table 40). On medium supplemented with BAP, flower buds at the end of experiment attained a size of 9.00-10.86 mm, petals measured 6.50-8.29 mm but anthers were only 2.00-3.67 mm long. The size of the pistil varied between 3.00-7.29 mm (Table 40). Low concentrations of BAP (10^{-12}-10^{-6}M) favoured better development of floral parts while higher

concentration (10^{-4}M) retarded their growth (Table 40). Although normal egg apparatus (Synergids and egg cell) was not evident, a large cell with prominent nucleus in the embryo sac of ovules of BAP treated flower buds was observed (Fig. 38.1c). The style in such floral buds besides revealing epidermis and cortex also revealed transmission tissue (Fig. 38.2c). The stigma was bilobed and comprised of an epidermis, parenchymatous stigmatic tissue and stigmatic papillae (Fig. 38.2c). However, pollen grains were not formed in the anther due to necrosis of anther tissue (Fig. 38.3c).

The effect of various concentrations of kinetin on in vitro development of flower buds of S. khasianum is given in Table 41 and Figs. 37.3A,a-F,f. At kinetin concentration 10^{-4}M maximum number of floral buds (27.27%) developed into anthesized flowers (Table 41). Higher or lower concentrations of kinetin reduced the ability of excised floral buds to develop into flowers (Table 41). Maximum elongation of floral buds was evident at 10^{-8} M of kinetin (Table 41). Floral parts such as petals, anthers and pistil, however, showed variable size (Table 41). Better development of floral parts was evident when higher concentrations (10^{-6} and 10^{-8} M) of kinetin were used (Table 41; Figs. 37.3C,c, D,d).

Similar to BAP, kinetin also induced better development of embryo sac revealing an organised egg apparatus inside the embryo sac **(Fig. 38.1d)**. The pistils of kinetin treated flowers exhibited well differentiated stylar tissue and stigmatic papillae **(Fig. 38.2d)**. But in anthers instead of pollen grains densely staining necrotic tissue was present inside the anthers locules **(Fig. 38.3d)**. In one case, where 10^{-8}M of kinetin was used, flowers developed parthenocarpic fruit after 50 days of culturing **(Figs. 38.3F,f)**.

Amongst the various growth hormones used, IAA induced better growth and development of floral buds **(Figs. 37.4)**. At lower concentrations of IAA (10^{-12} - 10^{-10}M) greater percentage of floral buds developed into near perfect flowers but at higher concentrations the percentage of floral explants developing into flower was lesser **(Table 42)**. At higher concentration of IAA (10^{-4}M), instead of flower development, root developement occurred **(Figs.37.4E,e)**. Maximum growth of floral buds was evident at 10^{-10}M of IAA and the floral buds measured 11.33 mm at the end of culturing **(Table 42; Figs.37.4B,b)**. However, other flower parts (petals, anthers and pistil) were smaller in comparison to the floral parts of flowers developed <u>in vivo</u> **(Table 42)**. IAA at 10^{-10}M concentration produced well developed flowers **(Figs. 37.4B,b)**. Embryo sac of IAA treated flowers contained two polar cells with

prominent nuclei (**Fig. 38.1e**). The pistil also revealed well differentiated stylar tissue (**Fig. 38.2e**), bilobed stigma containing parenchymatous stigmatic tissue and stigmatic papillae (**Fig. 38.2e**). Interestingly, 10^{-10}M and 10^{-8}M of IAA treated anthers contained well developed pollen grains of two types - (i) pollen with dense cytoplasm which stained intensely with saffranin and (ii) pollen in which cytoplasm stained lightly with saffranin. The pollen of later category could probably be sterile (aborted) pollen (**Fig. 38.3e**). However, even intensely stained pollen were smaller compared to pollen grains formed in vivo (**Figs. 38.3a & e**).

Amongst the growth hormones used NAA was least effective in supporting proper development of floral buds in vitro (**Table 43; Fig. 37.5A,a-E,e**). NAA at 10^{-10} M induced maximum number of floral buds developing into anthesized flowers (**Table 43**). However, development of flowers was better in medium having 10^{-8}M of NAA and the flowers so developed were abnormally bigger than flowers developing in vivo (**Table 43; Figs.37.5C,c**). Higher concentration of NAA (10^{-4}M) could not support development of flower. In this treatment the cultured floral buds developed into callus like structures (**Figs. 37.5E,e**). Petals of NAA treated flowers were greenish in colour (**Figs. 37.5A,a-D,d**). NAA treated flower buds measured between 10.00-12.50 mm, the

petals 9.00-11.00 mm, the anthers 1.50-3.00 mm while the pistil ranged between 6.00-7.00 mm **(Table 43).**

In the present study the stem segment attached to the inflorescence elongated to a considerable length before flowering. This was evident in all the growth hormone treated flower buds except NAA treated flower buds **(Figs. 37.2-37.5).**

DISCUSSION

The observations presented in this study show that considerable growth of S. khasianum flower buds of varying size can be achieved in (basal MS) (Murashige and Skoog, 1962) medium. However, all the three sizes of flower buds failed to attain normal development of floral parts in absence of growth hormones. Very young flower buds, although achieved considerable elongation, turned glassy and fragile. Growth in older flower buds was slow, nevertheless, better pistil growth was achieved. Such limited growth of excised young flower buds of tomato in absence of growth hormones was also observed by Rastogi and Sawhney (1986).

In presence of growth hormone (BAP, kinetin, IAA and NAA) the cultured flower buds not only produced the full complement of floral parts, but the differentiation of ovules and stylar tissue proceeded as in vivo. Lower

concentrations (10^{-12} - 10^{-8}M) of growth hormones nurtured better development of flower buds while higher concentrations (10^{-6} - 10^{-4}M) induced abnormal growth. Amongst the growth hormones tested, NAA was least effective in bringing out proper development of floral parts. High concentration of NAA (10^{-4}M) induced abnormal flower and callus like structures. The requirement of growth hormones for flower bud development under in vitro conditions has been demonstrated by many workers (Tepfer et al., 1963, 1966; Hicks and Sussex, 1970; Polowick and Greyson, 1982; Rastogi and Sawhney, 1986, The present findings that flower buds can be grown to anthesis in artificial medium supplemented with a single growth hormone is in agreement with the findings of Rastogi and Sawhney (1986, 1988). Brulfert and Fontaine (1967) also obtained normal flowers from excised flower buds of Anagallis arvensis in simple medium supplemented with IAA.

Hicks and Sussex (1970) and Rastogi and Sawhney (1986, 1988) demonstrated the essentiality of kinetin and BAP for the growth and development of floral organs to maturity. In the present study BAP or kinetin (cytokinin) could not induce formation of pollen grains in the anther. This is contradictory to the findings of earlier workers (Hicks and Sussex, 1970; Rastogi and Sawhney, 1986, 1988) who observed pollen grains development in

floral buds grown in kinetin supplemented medium. In the present study though low concentrations (10^{-10}- 10^{-8}M) of IAA induced development of pollen grains. The pollen grains appeared to be underdeveloped compared to pollen grains developed *in vivo*. At higher concentrations of IAA, no pollen development occurred. Blake (1966) observed that larger flower buds with pre-meiotic archesporial tissue tended to produce pollen more easily in *Viscaria* species. Thus differential potentiality of plant species to *in vitro* culture, growth hormone and developmental stage of the explants could be responsible for inducing pollen formation.

Present study also indicated that high concentration (10^{-4}M) of IAA induced root development on the elongated stem segment but inhibited flower bud development. Such root development due to high concentration of auxins is also reported in African violet (Vasquez and Short, 1978).

Consideration of the developmental stage of the explant, presence or absence of leaves at the time of inoculation, physical conditions during *in vitro* growth, and an appropriate nutrient medium are essential for normal development of flower buds (Konar and Kitchlue, 1982). In the present study although no attempt was made to find out the importance of stem segment on the excised flower buds, the normal flower development *in vitro* was

accompanied by considerable growth of the stem segment.

The present study was conducted with a view to investigate the role of various growth hormones in regulation of pistil development in S. khasianum. Earlier De Jong and Bruinsma (1974b) demonstrated that pistil development of Cleome iberidella was strongly stimulated by cytokinins. The results presented here indicated that variable length of pistil was obtained even with the same concentration of growth hormone.

Table 39. Effect of bud size on bud and pistil growth *in vitro* during 15 days in *S. khasianum*

Bud length at culture (mm)	Pistil length at culture (mm)	Bud length after culture (mm)	Pistil length after culture (mm)	Percent increase in elongation	
				Bud	Pistil
1.32±0.06	0.25	5.40±0.24	0.45±0.08	309.09±17.81	80.00±28.41
2.39±0.07	0.50±0.03	6.90±0.41	1.90±0.41	188.70±17.02	280.00±81.38
3.23±0.06	0.93±0.03	8.33±0.76	4.17±0.70	157.89±23.53	348.39±75.61

Table 40. Effect of benzylaminopurine (BAP) on *in vitro* development of excised flower of *S. khasianum*

Treatment		Concentration (M)	No. of buds cultured	No. of flowers after culture	Percentage of flower after culture	Floral organ (Length in mm)			
						Bud	Petal	Anther	Pistil
Control	In vivo	-	-	-	-	10.53±0.20	12.40±0.16	7.03±0.07	9.23±0.10
	In vitro	0	15	1	6.67	8.20	6.00	2.70	4.00
BAP		10^{-12}	17	3	17.65	10.86±0.46	8.29	3.50±0.61	7.29±0.52
		10^{-10}	12	3	25.00	9.25±0.59	6.88±0.55	3.11±0.39	6.00±0.76
		10^{-8}	15	3	20.00	9.85±0.26	6.50±0.56	3.67±0.56	5.83±0.60
		10^{-6}	16	2	12.50	9.83±0.79	8.00±0.58	2.29±0.26	7.14±0.39
		10^{-4}	15	1	6.67	9.00	7.00	2.00	3.00

± S.E.

Table 41. Effect of N^6-furfuryladenine (kinetin-KN) on *in vitro* development of excised flower bud of *S. khasianum*

Treatment		Concentration (M)	No. of buds cultured	No. of flower cultured	Percent of flower after culture	Floral organ (length in mm)			
						Bud	Petal	Anther	Pistil
Control	In vivo	-	-	-	-	10.53±0.20	12.40±0.16	7.03±0.07	9.23±0.10
	In vitro	0	15	1	6.67	8.20	6.00	2.70	4.00
KN		10^{-12}	10	1	10.00	8.00	7.00	3.00	6.00
		10^{-10}	12	1	8.33	10.00	7.00	3.00	6.00
		10^{-8}	11	3	27.27	10.33±0.88	7.00±0.58	2.83±0.17	8.00±0.58
		10^{-6}	10	2	20.00	10.00±2.00	8.50±1.50	3.00±0.00	7.00±2.00
		10^{-4}	11	2	18.18	9.00±0.29	7.00±0.17		3.17±0.17

± S.E.

Table 42. Effect of indole-3-acetic acid (IAA) on *in vitro* development of excised flower bud of *S. khasianum*

Treatment		Concentration (M)	No. of buds cultured	No. of flowers after culture	Percent of flower after culture	Floral organs (length in mm)			
						Bud	Petal	Anther	Pistil
Control	In vivo	-	-	-	-	10.53±0.20	12.40±0.16	7.03±0.07	9.23±0.10
	In vitro	0	15	1	6.67	8.20	6.00	2.70	4.00
IAA		10^{-12}	11	2	18.18	10.50±0.50	10.00±1.00	3.75±0.25	4.50±0.73
		10^{-10}	13	2	15.38	11.33±2.03	10.00±2.52	3.50±1.50	6.75±2.75
		10^{-8}	11	1	9.09	10.00	10.00	2.50	7.00
		10^{-6}	13	1	7.69	10.00	10.00	1.50	2.00
		10^{-4}	12	0	0	-	-	-	-

± S.E.

Table 43. Effect of a-naphthalene acetic acid (NAA) on *in vitro* development of excised flower bud of *S. khasianum*

Treatment		Concentration (M)	No. of buds cultured	No. of flower after cultured	Percent of flower after culture	Floral organ (length in mm)			
						Bud	Petal	Anther	Pistil
Control	In vivo	-	-	-	-	10.53±0.20	12.40±0.16	7.03±0.07	9.23±0.10
	In vitro	0	15	1	6.67	8.20	6.00	2.70	4.00
NAA		10^{-12}	6	1	16.67	10.00	9.00	3.00	6.00
		10^{-12}	5	1	20.00	10.00	9.50	3.00	6.00
		10^{-8}	6	1	16.67	12.00	10.00	2.00	6.00
		10^{-6}	8	1	12.50	12.50	11.00	1.50	7.00
		10^{-4}	7	0	0	-	-	-	-

± S.E.

Fig. 37.1 : Young excised flower buds of *S. khasianum* grown *in vitro* on MS basal medium devoid of growth hormones (control) 15 days after culture

A - in flask

a - outside flask

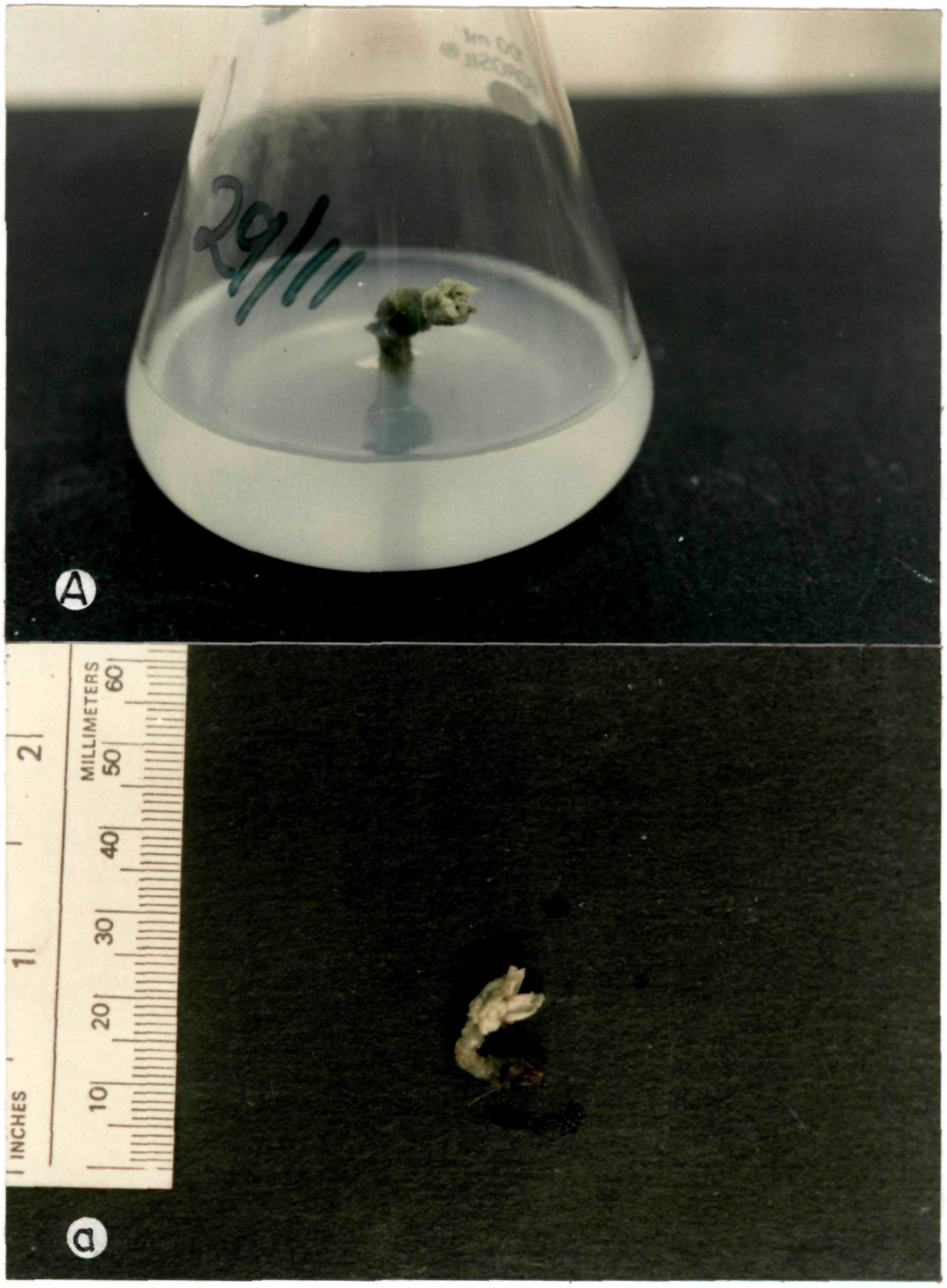

Fig. 37·1

Fig. 37.2 : Effect of BAP on the development of young excised flower buds of S. khasianum in vitro.

A,a - 10^{-12} M of BAP
B,b - 10^{-10} M of BAP
C,c - 10^{-8} M of BAP
D,d - 10^{-6} M of BAP
E,e - 10^{-4} M of BAP

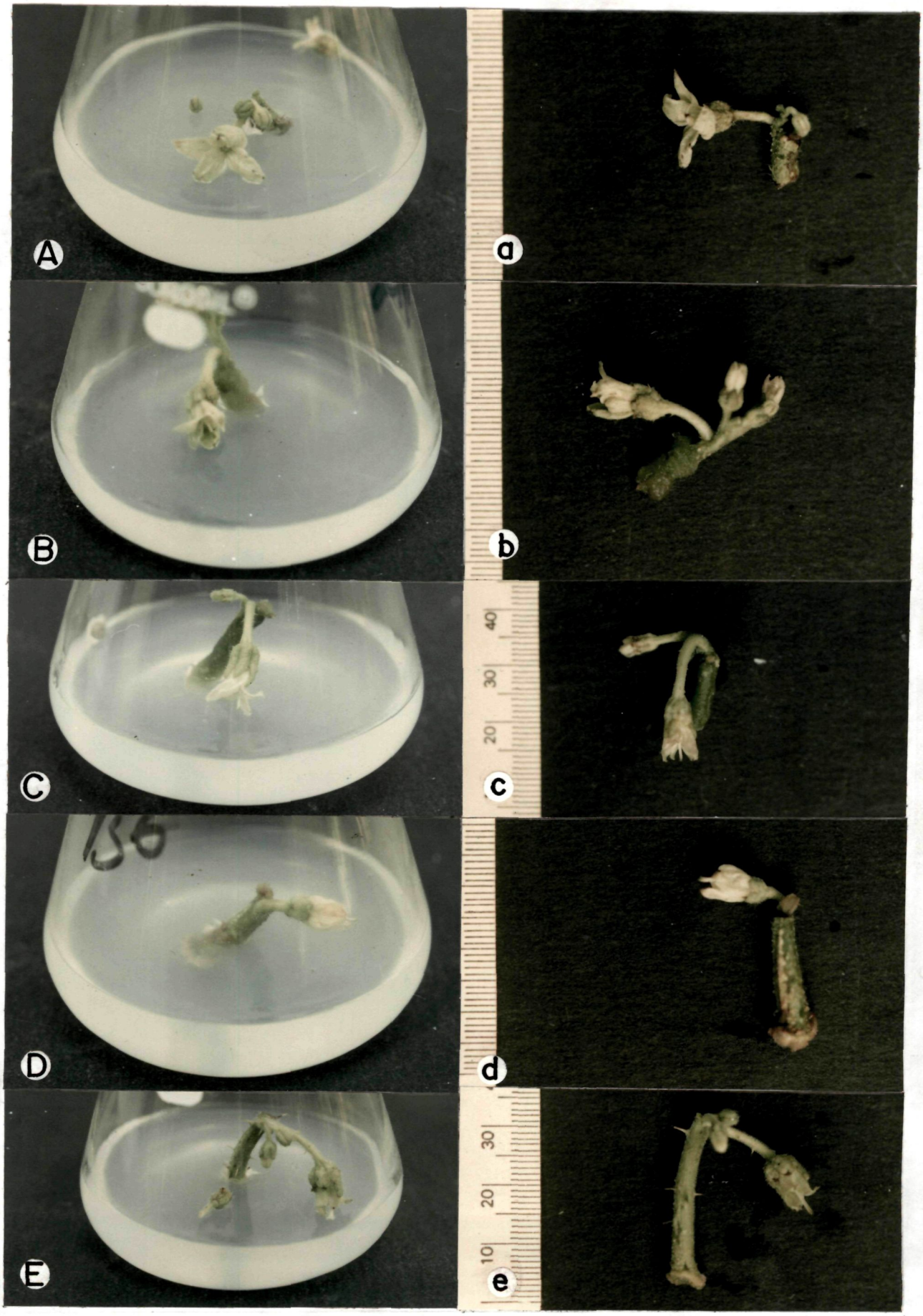

Fig.37·2

Fig. 37.3 : Effect of Kinetin on the development of young excised flower buds of S. khasianum in vitro.

A,a - 10^{-12} M of Kinetin

B,b - 10^{-10} M of Kinetin

C,c - 10^{-8} M of Kinetin

D,d - 10^{-6} M of Kinetin

E,e - 10^{-4} M of Kinetin

F,f - 10^{-8} M of Kinetin, 50 days after culture showing parthenocarpic berry formation.

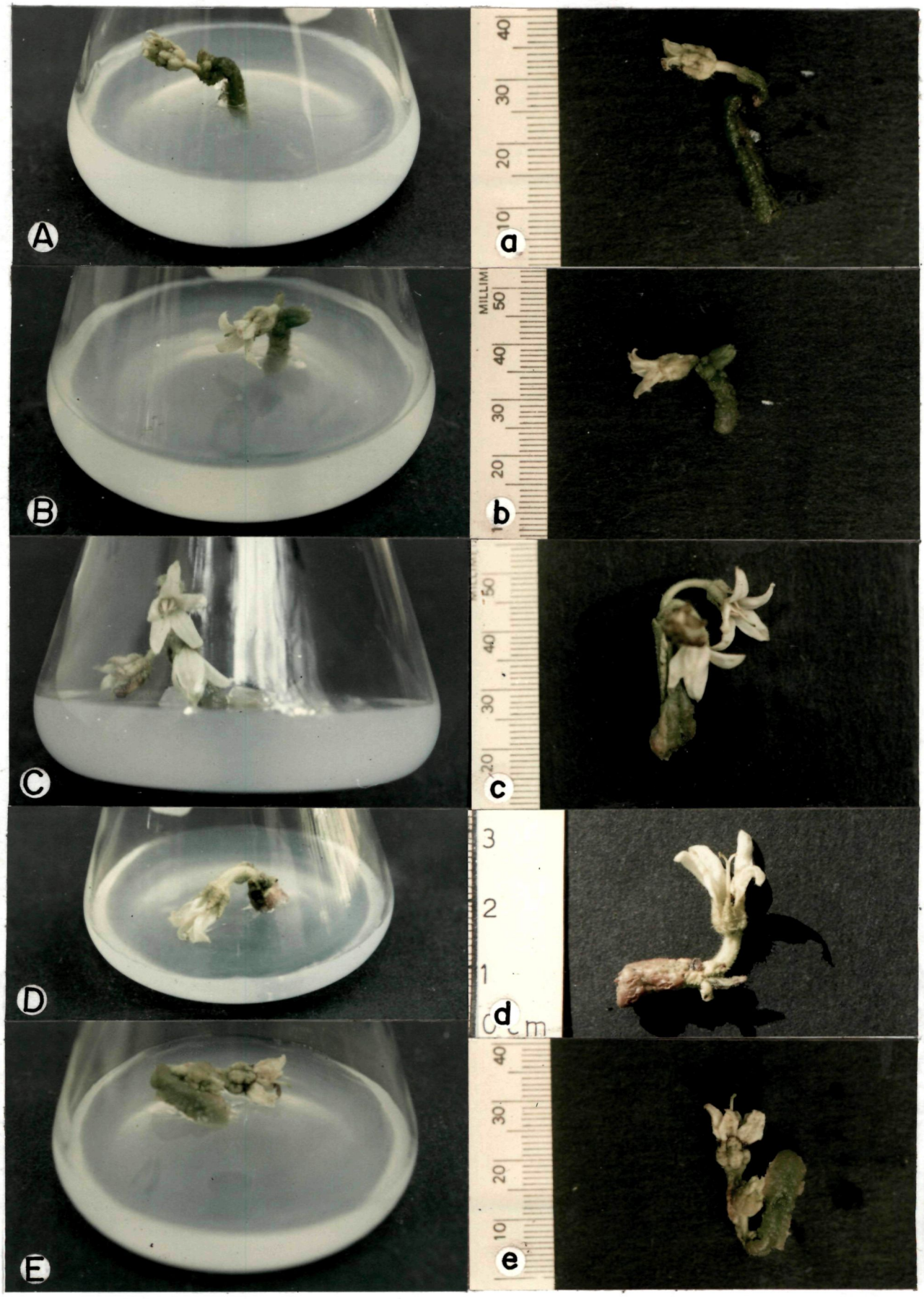

Fig. 37·3

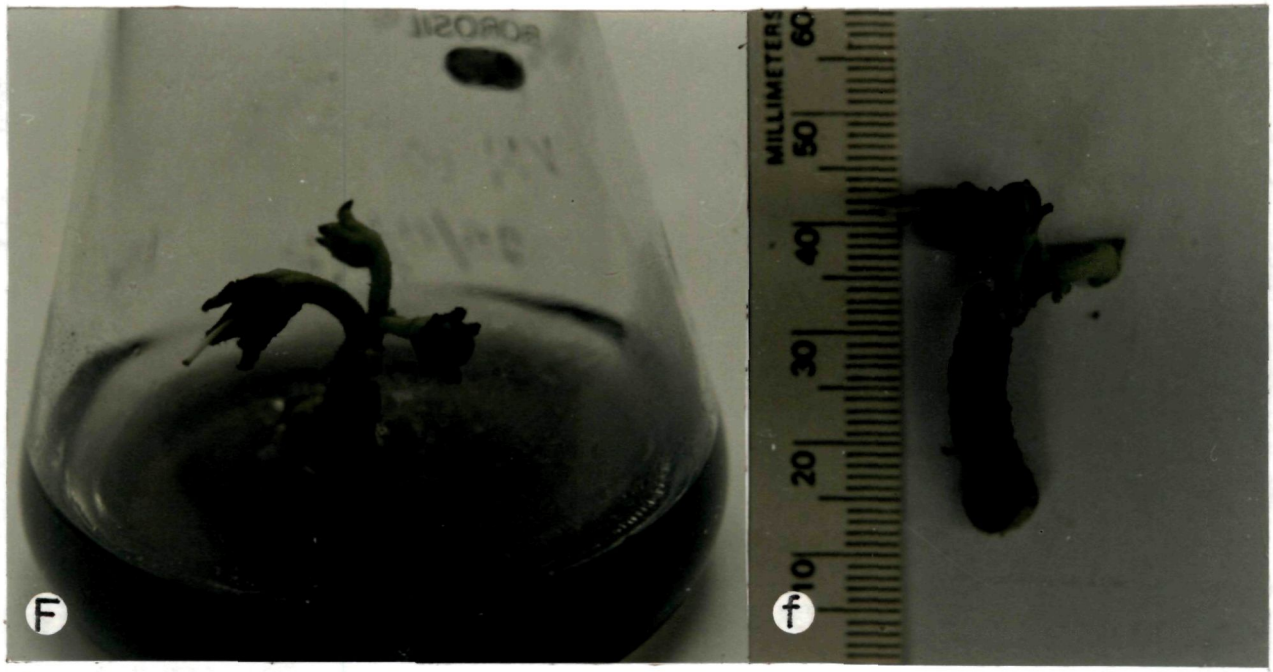

Fig. 37·3 Contd.

Fig., 37.4 : Effect of IAA on the development of young excised flower buds of S. khasianum in vitro.

A,a - 10^{-12} M of IAA

B,b - 10^{-10} M of IAA

C,c - 10^{-8} M of IAA

D,d - 10^{-6} M of IAA

E,e - 10^{-4} M of IAA, showing root formation on the explant.

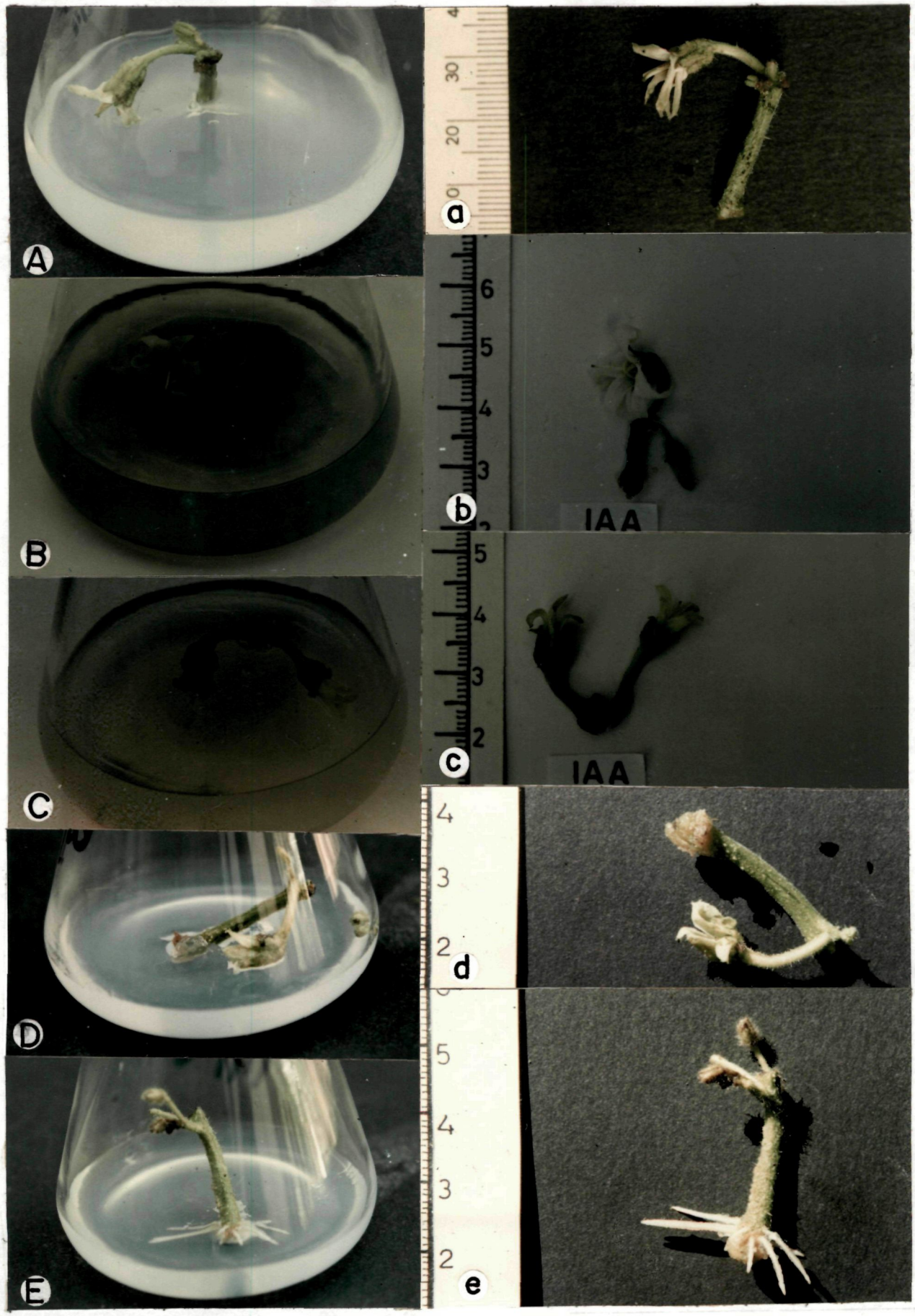

Fig.37·4

Fig. 37.5 : Effect of NAA on the development of young excised flower buds of S. khasianum in vitro.

A,a - 10^{-12} M of NAA
B,b - 10^{-10} M of NAA
C,c - 10^{-8} M of NAA
D,d - 10^{-6} M of NAA
E,e - 10^{-4} M of NAA

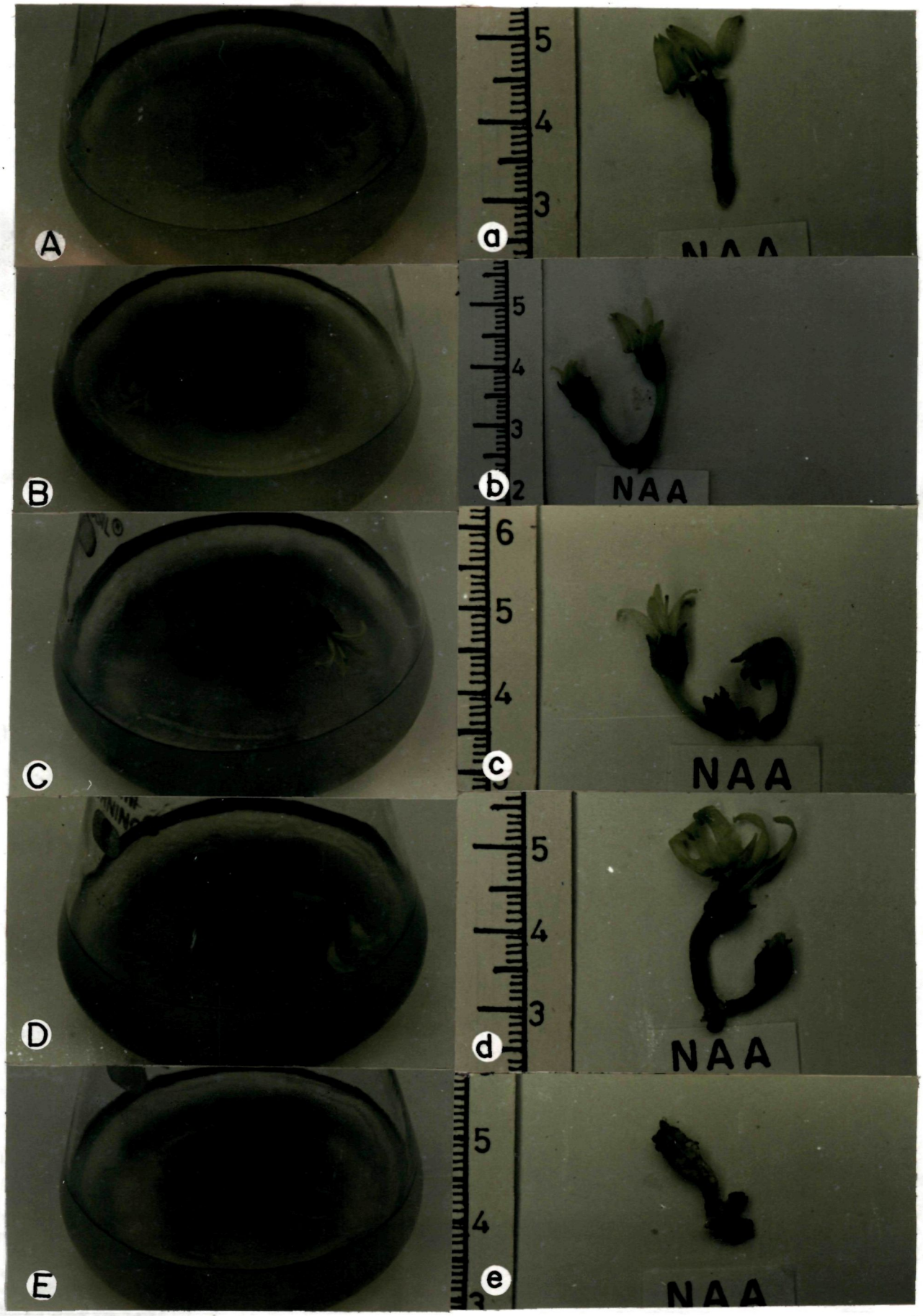

Fig. 37·5

Fig. 38.1 : Cross section of ovules from flowers formed on MS medium supplemented with/without growth hormones, 15 days after culture in *S. khasianum* (All figures **x 8,500**).

a - *In vivo* flower showing normal egg apparatus (**ea**)

b - Control flower (grown *in vitro* without growth hormone) showing arrested development of female gametophyte

c - BAP (10^{-10}M), showing large cells (**lc**) with prominent nucleus in the embryo sac

d - Kinetin (10^{-8} M) showing egg apparatus (**ea**) development

e - IAA (10^{-10} M) showing polar nuclei (**pn**)

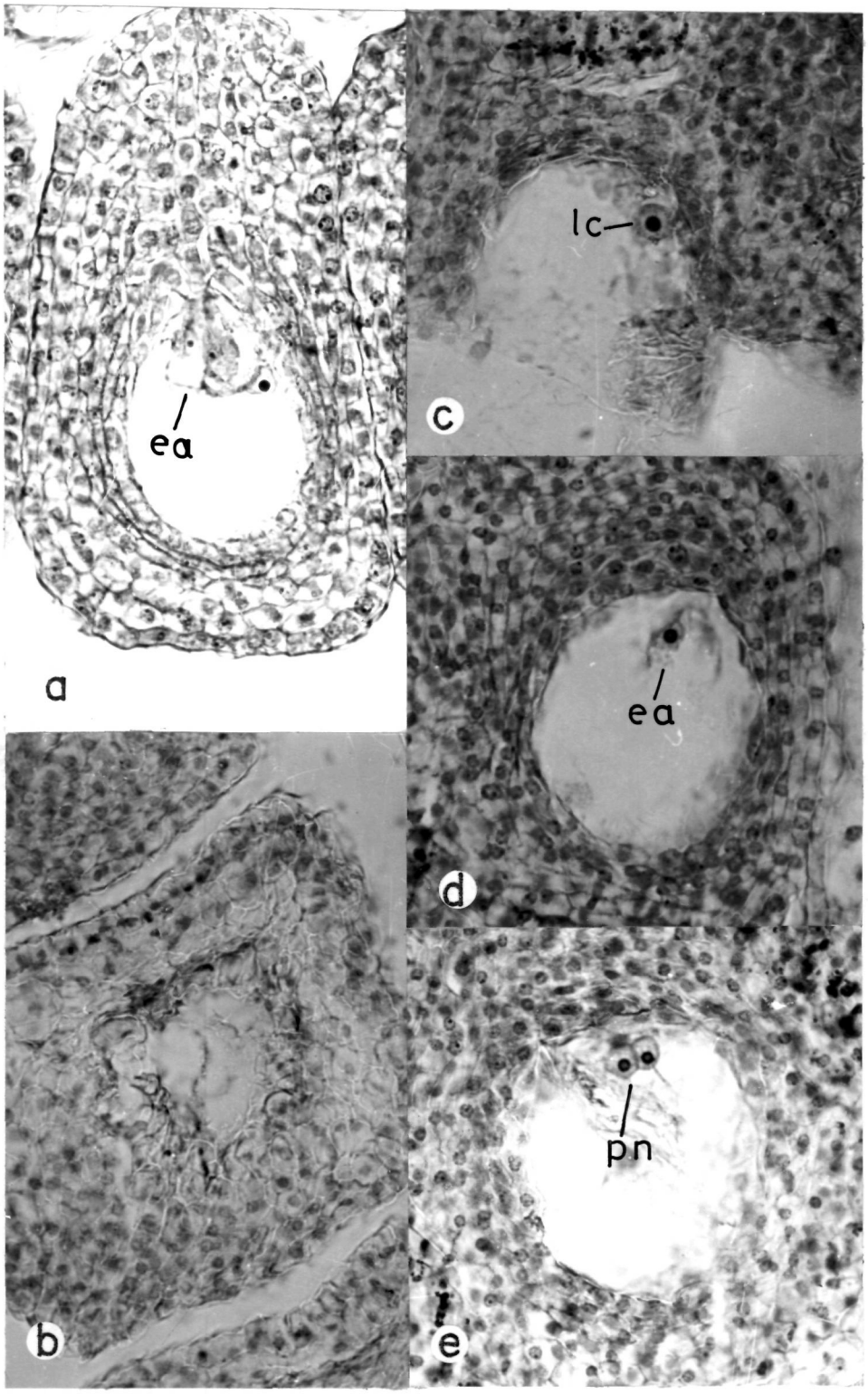

Fig. 38·1

Fig. 38.2 : Longitudinal section of pistil of *in vivo* and *in vitro* flowers of *S. khasianum* (All figures x 2,720 except a x 1,338.75 and b x 2,125).

a - *In vivo* pistil

b - *In vitro* control pistil with arrested growth

c - BAP (10^{-10} M) pistil

d - Kinetin (10^{-8} M) pistil

e - IAA (10^{-10} M) pistil

tt - transmission tissue

sp - stigma papillae

sp(g) - stigma papillae with pollen grains.

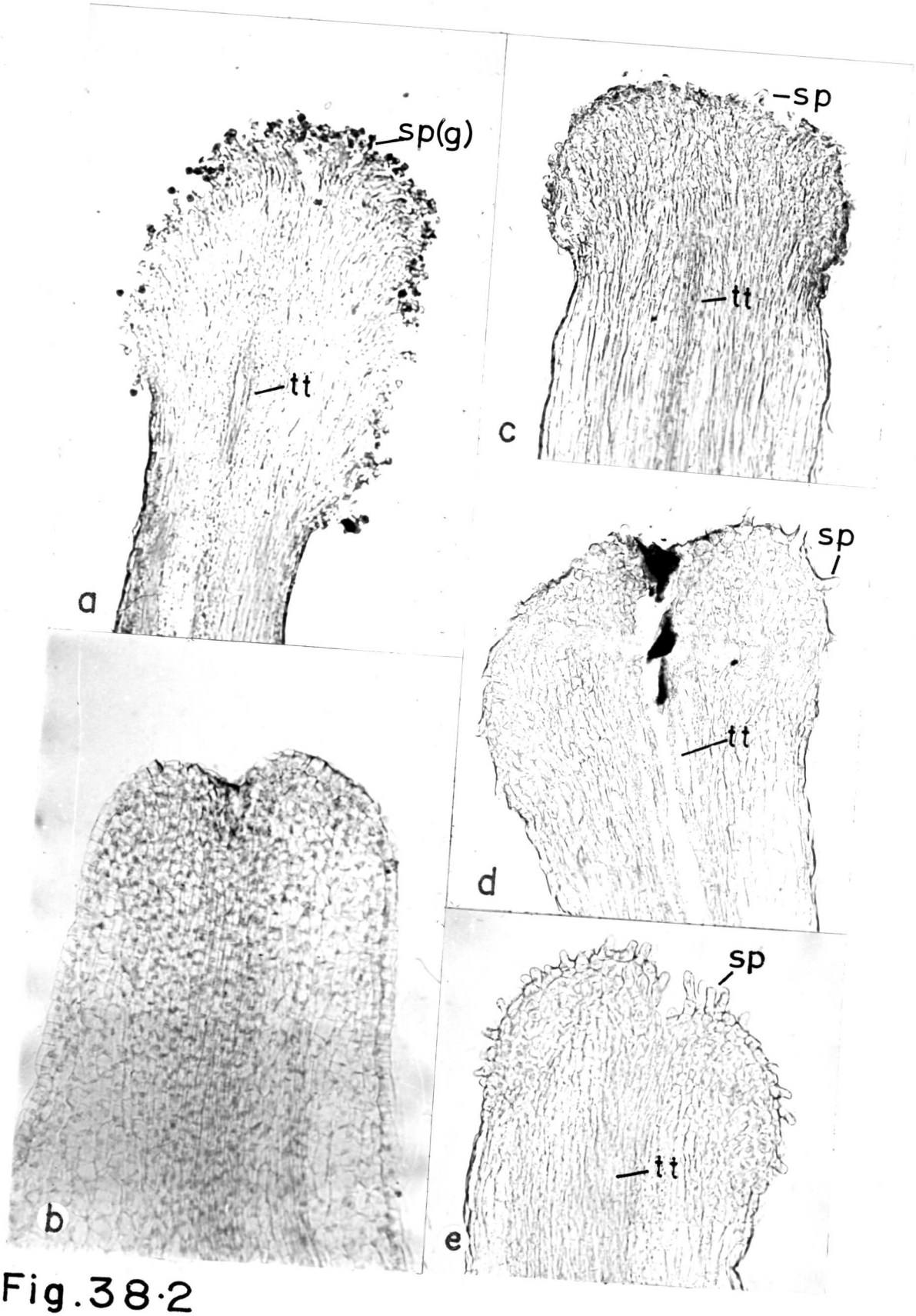

Fig.38·2

Fig. 38.3 : Longitudinal section of anther.

a - Anther developed *in vivo* showing pollen (X 4,250)

b - Anther developed *in vitro* on MS medium devoid of growth hormones showing aborted anther chamber (x 2,176)

c - Anther developed *in vitro* on MS medium supplemented with BAP (10^{-10} M) showing aborted anther chamber (x 1,700)

d - Anther developed *in vitro* on MS medium supplemented with Kinetin (10^{-8} M) showing aborted anther chamber (x 1,700)

e - Anther developed *in vitro* on MS medium supplemented with IAA (10^{-10}M) showing pollen grains (x 8,500)

pg - pollen grains.

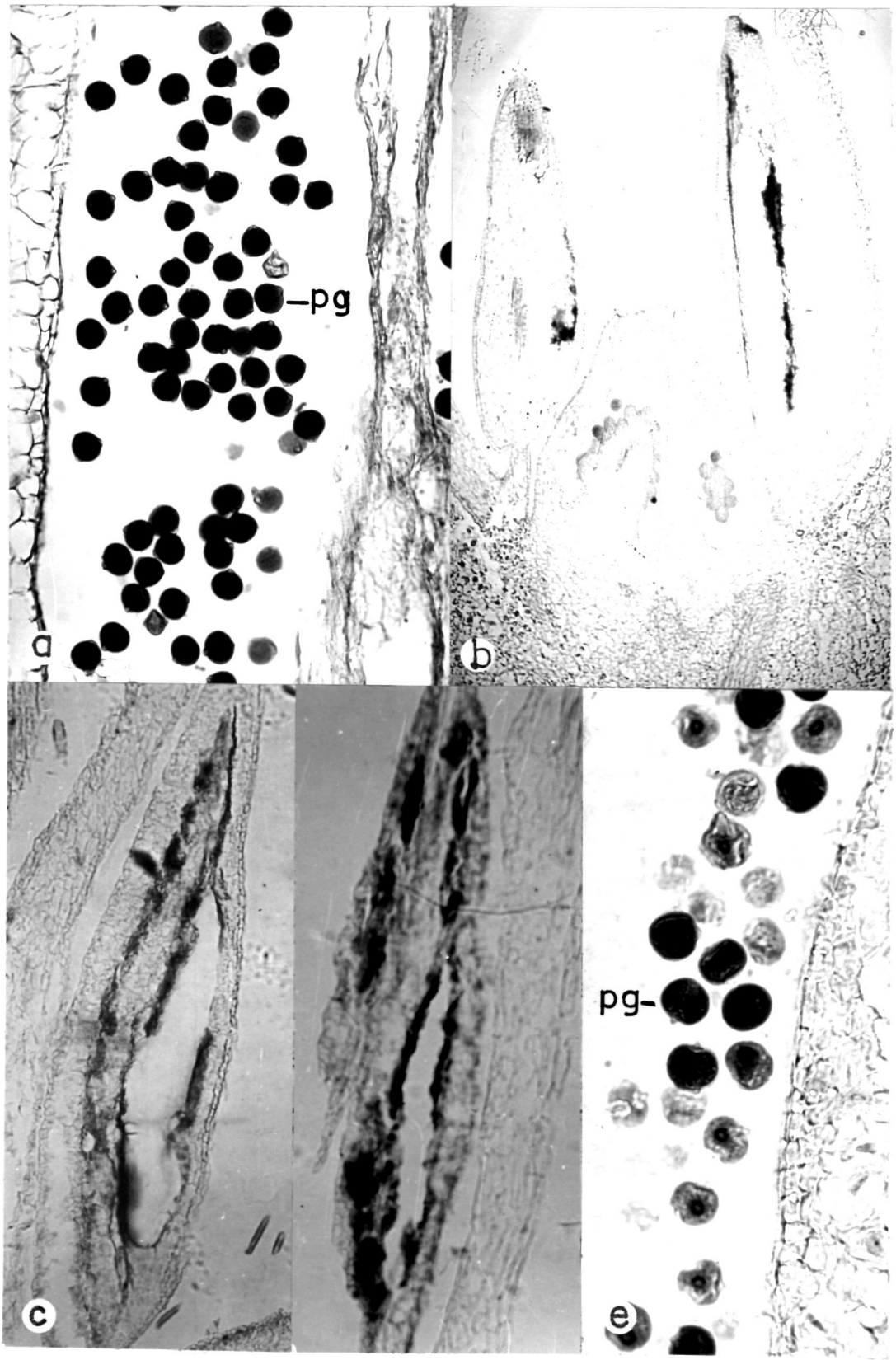

Fig. 38·3

Chapter-VII

GENERAL DISCUSSION AND CONCLUSIONS

Pollen of Solanum khasianum and S. marginatum although germinated in a wide range of pH (5.0-7.5) optimal pollen germination and pollen tube elongation in both the species occurs at pH 6.5. Thus pH requirements of S. khasianum and S. marginatum pollen resemble Vinca rosea pollen which also requires 6.5 pH for optimal germination and tube elongation (Bandyopadhya and Mukerjee, 1977).

Temperature influenced pollen germination and pollen tube elongation in S. marginatum. 30°C optimised pollen germination, tube elongation and development of long pollen tubes. Temperatures lower and

higher than 30°C were detrimental to pollen tube elongation and increased development of short pollen tubes. High temperature (35°C) induced bursting of pollen in large number. High temperature causing excessive swelling of pollen tubes leading to profuse bursting of pollen has also been reported in a number of other plant species (Smith, 1942; Vasil and Bose, 1959; Johri and Vasil, 1961).

S. marginatum pollen require 15% of sucrose for optimal pollen germination, pollen tube elongation and development of long pollen tubes. In absence of sucrose in the germinating medium profuse bursting of S. marginatum pollen occurred. De Bruyn (1966) also observed that absence of sugar in the germinating medium caused bursting of Setaria sphacelata pollen. Sucrose regulates pollen germination and pollen bursting by providing nutrition to germinating pollen and by serving as an osmoticum (Johri and Vasil, 1961).

Boron was beneficial for germination and tube elongation of S. marginatum pollen. Significant stimulation of pollen germination over control occurred in a wide range of boron concentration (100-400 ppm). Boron requirement for pollen tube growth, however, was limiting. Significant enhancement of pollen tube growth over control was evident in medium having 100-200 ppm of boron. 200

ppm of boron supported the occurrence of long pollen tubes in larger number than other concentrations of boron. Higher concentrations (300-400 ppm) did not show any adverse effect on pollen tube growth vis-a-vis control but increased the occurrence of short pollen tubes. Thus optimum boron requirement of S. marginatum pollen resembled pollen of other solanums investigated by Ravindran and Chauhan (1980). Stanley and Linskens (1974) stated that plants requiring high boron for in vitro pollen germination have high endogenous boron. Boron content of S. marginatum pollen, therefore, appears to be high.

Pollen of S. khasianum and S. marginatum responded differently to exogenous supply of calcium. S. khasianum pollen required exogenous supply of calcium (10 ppm) but pollen of S. marginatum did not require any calcium for germination and tube growth. High concentrations of calcium was detrimental for pollen of both the species. Pollen rich in endogenous calcium do not require exogenous supply of calcium for germination and tube growth (Shivanna and Johri, 1985). Thus in the present study, pollen of S. khasianum requiring small quantity of calcium and S. marginatum pollen not requiring calcium could be due to higher calcium content in their pollen.

Pollen germination and tube elongation of S. khasianum was inhibited by exogenous potassium. The degree of inhibition increased with the increasing concentration of potassium. In S. marginatum, however, potassium stimulated pollen germination. Maximum pollen germination occurred at 200 ppm of potassium. Pollen tube elongation of S. marginatum although, was insensitive to potassium, higher frequency of long pollen tubes occurred at 200 ppm of potassium. Thus pollen of S. khasianum and S. marginatum differ in their requirement of potassium for germination and tube growth.

Gelling of the medium with 1% of bacto-agar was beneficial for pollen of S. khasianum and S. marginatum. Higher concentrations of agar, however inhibited pollen germination and pollen tube growth in both the species. Improved pollen germination and tube growth due to gelling occurs in maize also (Cook and Walden, 1965). During pollen germination imbibition or hydration phase is important and gelling of the medium is known to regulate hydration of the pollen (Shivanna and Johri, 1985). Thus in the present investigation controlled hydration through gelling of the medium could be the reason for stimulation of pollen germination and tube elongation in S. khasianum and S. marginatum.

Germination of S. khasianum or S. marginatum pollen was stimulated by 200 krad of gamma rays but higher doses of radiation (500-800 krad) inhibited pollen germination. The degree of inhibition increased with the increasing dosage of radiation. The LD_{50} dose for germination of S. khasianum (600-700 krad) and S. marginatum (500-600 krad) though differed, 800 krad dose proved LD_{100} for pollen of both the species. Unlike pollen germination, pollen tube elongation of S. khasianum and S. marginatum was inhibited by 50 and 200 krad of radiation respectively. Thus in both the species, compared to the radiation dose required to inhibit pollen tube elongation, relatively higher doses of irradiation were required to inhibit pollen germination suggesting that pollen germination and tube elongation are two different processes. Similar observations were made by Cuny and Roudot (1991) in melon pollen. The high radioresistance of S. khasianum and S. marginatum pollen was indicated by high LD_{50} value.

Kinetin, IAA and GA_3 influenced pollen germination and pollen tube elongation in S. khasianum and S. marginatum. Pollen of S. khasianum was insensitive to low concentration (1 ppm) of kinetin whereas low concentration (1 ppm) of kinetin inhibited both pollen germination and tube elongation in S. marginatum. Compared to S. marginatum, pollen of S. khasianum required higher

concentration of IAA to elicit inhibition. Similarly, pollen of S. khasianum was insensitive to low concentrations (1-10 ppm) of GA_3 while germination of S. marginatum was inhibited by 1 ppm of GA_3. However, pollen tube elongation of S. marginatum was not influenced by 1-25 ppm of GA_3. These results suggest differential sensitivity of the two species to kinetin, IAA and GA_3. Similar observations were made by Ravindran and Chauhan (1986) in S. khasianum and S. indicum. These growth hormones inhibit pollen germination and pollen tube elongation in S. sisymbrifolium as well (Kuruvilla et al., in press).

In the present study a correlation existed between growth hormones-induced responses of pollen tube elongation and cytochemical constituents of the pollen tubes. Lesser utilization of insoluble polysaccharides and protein was associated with inhibition by kinetin while RNA was not influenced by kinetin in pollen tubes of both the species. Similarly, IAA caused lesser utilization of insoluble polysaccharides in pollen tubes of the two species. IAA (10 ppm) had no apparent effect on protein in pollen tubes of S. marginatum while protein was reduced in tubes of S. khasianum pollen. IAA treated pollen tubes of S. khasianum showed decreased RNA in the apical region while sub-apical region of S. marginatum revealed an

increased intensity of RNA. IAA had no influence on RNA in other regions of pollen tubes in the two species. These findings further suggest the differential response of the pollen of two species investigated to IAA. IAA induced decreased protein in the pollen tubes of S. khasianum could be due to excessive dissociation of protein caused by IAA leading to tube growth inhibition as put forward by Northen (1942). Although GA_3 (10 ppm) did not influence pollen tube elongation of the two species it induced increased insoluble polysaccharides (sub-apical region) and reduced protein (throughout) with no effect on RNA in S. khasianum while in pollen tubes of S. marginatum insoluble polysaccharides, protein and RNA (apical region) was not affected but RNA showed increased intensity in the sub-apical and distal region. Thus pollen of the two species differ in their sensitivity to GA_3. Growth hormones inhibition of pollen tube elongation through lesser utilization of insoluble polysaccharides, protein and RNA is recorded in Pinus kesiya (Katiyar, 1991).

Pollen of S. khasianum and S. marginatum irradiated with their respective LD_{50} dose of gamma rays inhibited utilization of insoluble polysaccharides, protein and RNA. Such inhibition of cytochemical constituents utilization due to gamma irradiation was observed in S. torvum by Chauhan and Katiyar (1990). Growth hormones (kinetin and IAA)

further sensitized radiation injury to pollen tube elongation in the two species through further decrease in utilization of insoluble polysaccharides, protein and RNA in the two species. GA_3 did not induce any statistically significant influence on pollen tube elongation of irradiated pollen in the two species but cytochemical constituents were affected. Compared to irradiated control pollen tubes in 600 krad + 10 ppm of GA_3 treated pollen tubes of S. khasianum insolublle polysaccharides and protein was not affected but an increase in RNA (apical region) comparable with the unirradiated pollen tubes was evident. Such may be the reason for partial recovery of irradiated S. khasianum pollen germination due to GA_3 treatment. Radiosensitizing effect of growth hormones on irradiated pollen by lesser utilization of insoluble polysaccharides and protein are reported in Pinus kesiya (Katiyar, 1991).

In both the species, irradiated pollen reduced berry forming ability. The degree of berry setting decreased with the increasing dose of gamma radiation. Reduced fruit setting due to pollination with irradiated pollen is also reported in other plant species (Sniezko and Visser, 1987; Kgazal, 1989; Chauhan and Katiyar, 1990).

Berries formed from pollination with variously irradiated pollen matured simultaneously. In S. khasianum berries formed from irradiated pollen were of smaller size and lesser weight compared to control berries. But berry size and weight in S. marginatum was not affected.

Number of seeds produced per berry in both the species was greatly reduced due to pollination with irradiated (5 krad) pollen. Seeds produced in berries formed as a result of irradiated pollen (5 krad) also weighed less and contained considerable amount of empty/chaffed seeds. Pollen irradiated with higher doses (25-500 krad) of gamma rays induced seedless berries in S. khasianum. According to Nitsch (1965) seeds developing in a fruit have a bearing on its development and growth; fruits having less seeds exhibit reduced growth and there is a linear relationship between developing seeds and weight of fruit. Therefore, reduce size and weight of berries in the present study may also be due to adverse development and growth of seeds induced by irradiated pollen. Pollen irradiation-reduced berry growth and seed development has also been observed in S. torvum (Chauhan and Katiyar, 1990). Development of seedless fruits due to pollination with irradiated pollen had been reported in other plant species, e.g., pear (Sniezko and Visser, 1987) and date palm (Kgazal, 1989).

Inhibition of seed development as a result of pollination with irradiated pollen is clearly displayed in dissected/sectioned ovules. In both the species, pollen irradiated with 5 krad of gamma rays did not hamper fertilization. However, some of the embryos degenerated in the later stages of development. This appears to have caused empty/chaffed seeds in the berries. In S. khasianum although pollination with pollen, irradiated with 25-200 krad of gamma rays did not induce any change in the embryo sac at the beginning, endosperm development was delayed. Enlarged proliferated endothelial cells were also observed in these ovules. These proliferated cells probably digested the endosperm and caused the death of the zygote/embryo in its early stage of development. This may be the reason for absence of seeds in its rudimentary form. Such proliferating tumorous tissue of endothelium leading to abortion of embryos had been reported in literature (Lee and Cooper, 1958; Rappaport, 1965). In those ovules pollinated with 350-500 krad irradiated pollen endothelial cells collapsed early and the egg cell enlarged indicating that fertilization did not occur. However, pollination in these ovules acted as stimulus and induced parthenocarpic berries. In S. marginatum ovules

pollinated with 25 and 50 krad irradiated pollen tubes entered ovule but the egg/zygote appeared disorganised and disintegrating. The ovule as a whole collapsed within one week of pollination. Thus these ovules failed to induce fruiting. According to Vassileva-Dryanovska (1966), with an increase of the dose there is an increase in the damage of the male chromatin, but the pollen tubes are capable of growing to the embryo sac also at a very high doses. This phenomenon can be interpreted in view of the knowledge that the RNA and RNA-protein (in which the cytoplasm of pollen is rich) are much more resistant to radiation than DNA (in which the generative nucleus is rich) (Vassileva-Dryanovska, 1966). Therefore, it appears that although pollen tubes of high dose irradiated pollen of S. marginatum were capable of reaching ovules, the ovules became disorganised and collapsed leading to failure of fruiting because male chromatin may be too damaged to affect fertilization as suggested by Vassileva-Dryanovska (1966).

Germination of seeds formed as a result of 5 krad irradiated pollen of S. khasianum was reduced. Rate of germination also decreased in these 5 krad seeds. Such inhibition of seed germination and delayed germination of seeds formed from irradiated pollen was reported in Betula nigra, Oryza sativa and date palm (Clausen, 1973a; Chin and Gordon, 1989; Kgazal, 1989). Seedling abnormalities

such as varying number of cotyledons and varying leaf shape were produced in germinants from seeds produced from irradiated pollen. Similar observations were also made by earlier workers (Clausen, 1973b). Based on the above abnormalities, 5 krad plants could be categorised into various phenotypes; phenotype I having dicotyledon normal leaf type, phenotype II-dicotyledon triangular leaf type, phenotype III-dicotyledon roundish leaf type and phenotype IV-tricotyledon all fused together at the base. According to Chauhan (1978) and Ravindran (1981) leaf length is more sensitive to gamma radiation than leaf breadth in S. khasianum plants raised from irradiated seeds. However, present investigation revealed that leaf breadth was more sensitive to gamma radiation than leaf lenth in various phenotypes. Thus seed irradiation and pollen irradiation differ in their effect on leaf expansion. Number of spines on leaves of phenotypes I, III and IV were not affected while it was reduced in phenotype II. However, phenotypes III and IV were more spiny while spine frequency in phenotypes I and II was not affected. Thus, increase in spine intensity in the present study was due to reduction in leaf size. The effect of radiation on spine intensity through leaf expansion inhibition was also reported in S. khasianum raised from irradiated seeds (Ravindran, 1981). Thus the effect of gamma radiation on spine numbers and spine intensity in S. khasianum raised

from seed irradiation or pollen irradiation basically remain the same.

In the present investigation photosynthetic pigments were also influenced in various phenotypes. Chlorophylls showed varied response while carotenoids were always stimulated in all the phenotypes. These phenotypes are, therefore, more effective in photosynthesis. These observations supported earlier reports that chlorophylls are affected as a result of ionizing radiation (Reddy et al., 1984).

Present investigation also revealed that total number of flowers per plant, number of flowers per cluster or number of flower cluster per plant was also affected in one or the other 5 krad phenotypes of S. khasianum. Radiation-induced damage of various phenotypes was also evident in reduced flower size and deformed flowers such as varying number of sepals and petals, abnormal stamen and modification of pistil. Such deformities has been reported by earlier workers (Brewbaker and Emery, 1962; Chin and Gordon, 1989). Such abnormalities are known to be associated with cytological aberrations (Brewbaker and Emery, 1962). Pollen stainability in these phenotypes, except phenotype I_3 was also greatly reduced. Pollen from these phenotypes failed to induce any fruiting when selfed. However, pistil in all the phenotypes was fertile

and set berries. Thus these phenotypes were male sterile. Pollen sterility could be an index of radiation-induced genetical damage in plants (Brewbaker and Emery, 1962).

Interestingly, production of long-styled flowers in these phenotypes (II, III and IV) was greatly enhanced. In phenotype IV all the flowers produced were long style. Enhanced production of long-styled flowers as a result of seed irradiation with low dose of gamma rays has been reported in literature (Chauhan and Ravindran, 1979; Ravindran, 1981). The mechanism by which ionizing radiation controls flower production is however, not clearly understood (Gunckel and Sparrow, 1961).

As reported by other workers (Gunckel and Sparrow, 1961; Brewbaker and Emery, 1962; Sax, 1963) reduced plant height, inhibition of apical dominance, stimulation of lateral branches and spread at plant maturity was also observed in 5 krad phenotypes of S. khasianum. Although a higher frequency of long-styled flowers was observed in these phenotypes, total number of berries per plant formed was reduced. This may be due to radiation-induced early abscission of flowers (Gunckel and Sparrow, 1961; Brewbaker and Emery, 1962).

Berry size, berry weight and number of seeds per berry was also greatly reduced in these 5 krad phenotypes. However, weight of seeds was not affected. An increase of

moisture content over control was observed in berries of phenotype II, III and IV. Thus pollen irradiation with gamma rays increased variability in S. khasianum.

Young flower buds of S. khasianum grown in vitro on MS basal medium elongated considerably, however the growth is limited in absence of growth hormones. Maximum increase in elongation of flower buds occurred in young flower buds having a size of 1.32 mm, but these buds were abnormal and became glassy and fragile. Although percent increase in elongation of flower buds was reduced in bigger flower buds pistil growth was stimulated in these buds (3.23 mm).

Flower buds cultured in absence of growth hormones remained underdeveloped and abnormal. Incorporation of growth hormones - BAP, kinetin, IAA and NAA into the medium improved growth and development of excised flower buds. Low concentrations of growth hormones supported better growth of cultured floral bud, differentiation of floral organs and development of normal full bloom flowers. High concentrations of growth hormones induced abnormally developed flowers and retarded the growth, differentiation and development of floral parts. High concentration (10^{-4} M) of IAA induced root development on the explants. Growth hormones (BAP, kinetin and IAA) also improved the development of ovules and the stylar tissue. BAP and kinetin failed to initiate pollen formation in the

anthers while well developed pollen grains were observed in IAA treated flowers. This observation is in contradiction to the findings that cytokinins induce pollen formation while auxins inhibit its formation (Hicks and Sussex, 1970; Rastogi and Sawhney, 1986, 1988). This may be due to differential response/potentiality of the explants and species to in vitro culture condition, growth hormone and developmental stage of the explant. Growth hormones failed to elicit any conclusively consistent effects on elongation of pistil in the present study. Both short and long styles were produced with the same growth hormone at the same concentration. Developmental stage of the explant may be responsible for such variable response. It is also speculated that the stem segment attached to the inflorescence at the time of culture elongated as a result of growth hormone treatment and provided necessary factors/balance of growth hormones in response to the exogenous supply of growth hormones for the growth and development of floral organs as observed by other worker (Blaȼke, 1969) in Viscaria species which requires a pair of leaves at the time of culture.

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ZHANG, X. 1990. Development of male sterile water melon line and its utilization. XXIII Int. Hort. Congr., Italy. Abstract. pp. 91.

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APPENDIX

List of Publications

1. Belho, V. and Chauhan, Y.S. 1990. Effects of growth hormones and gamma radiation on pollen germination and pollen tube growth of Solanum marginatum L.f. Geophytology **19**(2): 147-150.

2. Belho, V. and Chauhan, Y.S. 1990. Effects of gamma radiation, growth hormones and modulation of radiation responses by phytohormones in Solanum khasianum Clarke. Phytomorphology **40**(1 & 2): 27-32.

3. Chauhan, Y.S. and Belho, V. 1990. Effects of gamma radiation on in vitro pollen germination, pollen tube growth and fertilizing ability of irradiated pollen in Solanum khasianum Clarke. Presented in 'Seminar on Pollination Biology'. Haryana Agricultural University, Hisar, and accepted for publication in **Progress in Pollination Biology.**

4. Chauhan, Y.S. and Belho, V. 1992. Irradiated pollen a tool for the improvement of Solanum khasianum Clarke. Communicated to 'First World Congress on Medicinal and Aromatic Plants for Human Welfare (WOCMAP)', Maastricht, Netherlands.