RADIOBIOLOGICAL STUDIES IN SOLASODINE YIELDING SOLANUMS

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I certify that the thesis entitled "RADIOBIOLOGICAL STUDIES IN SOLASODINE YIELDING SOLANUMS" submitted by S. Ravindran 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. He has been duly registered and 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.

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DEDICATED TO THE
LOVING MEMORY OF
MY FATHER

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CHAPTER I

INTRODUCTION

Steroidal hormones and corticosteroids have assumed great importance as they are used for various human ailments such as rheumatoid arthritis, addison's disease, chronic cases of asthma, leukaemia and obesity. They are also the active principles of oral contraceptives.

Several natural plant sources have been discovered which yield intermediates for the synthesis of steroidal drugs. Diosgenin, a steroid sapogenin obtained from *Dioscorea* has been commercially exploited for this purpose. Diosgenin can be converted into 3β- acetoxy - $\Delta 5$ - 16 pregnadiene - 20 - one, a key intermediate for the synthesis of steroidal drugs. The same intermediate has been obtained from solasodine (a steroidal alkaloid found in some *Solanum* spp.) which is a nitrogen analogue of diosgenin (Sato *et al.*, 1951). The realization that the glycoalkaloid solasodine is a suitable alternate raw material for the production of steroid hormones led to a worldwide search for the identification of *Solanum* species rich in solasodine content and suitable for large scale cultivation. Though a number of *Solanum* species are reported to yield solasodine in appreciable quantities, only few species occur wildly in India. Amongst these species, *Solanum khasianum* and *Solanum indicum* yield solasodine in commercially exploitable amounts (Maiti *et al.*, 1964; Chopra and Kapoor, 1968). However, commercial cultivation of these species have met with limited success because of low berry production resulting in reduced alkaloid yield. Furthermore, Ailments

1. Low [illegible] production
2. Existence of sharp spines on aerial parts
3. Asynchronous flowering

the presence of sharp spines on the aerial parts of the plant and asynchronous flowering poses a serious problem in the harvesting of berries. Therefore the need for an intensive breeding programme to improve solasodine yielding solanums has been emphasised, but the attempts to improve S. khasianum by hybridization have not succeeded (Kaul and Zutshi, 1977). Induction of tetraploidy in this species also did not prove to be promising (Kaul and Zutshi, 1977). Thus the possibilities to improve this species through conventional methods of plant breeding are meagre. Similar were the conclusions of Rudolph (1972) in the case of forest tree species exhibiting poor hybridization possibilities. Induced mutations are considered an alternative to hybridization and recombination in plant breeding (Brock, 1971). 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; Kaul and Kak, 1975; Kaul and Choudhury, 1975; Gupta et al., 1979). Gamma rays have been used for the genetic improvement of S. khasianum but the attempts have met with limited success (Bhatt, 1972; Chauhan et al., 1975a, b, 1976). For a successful crop improvement programme using ionizing radiations a thorough understanding of the radiobiology of the species used 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. As radiobiological studies in S. khasianum and S. indicum are lacking the present study was undertaken to make a comparative study of radiation responses in M_1 generation since this may provide basic information needed for irradiation experiments. The following aspects were considered:

1. Seed germination and seedling survival.
2. Growth and yield.
3. Shoot apical meristem.
4. Flowering responses.
5. Pollen germination and pollen tube growth.
6. Heterostyly.

CHAPTER II

REVIEW OF LITERATURE

Radiation:

The nature and frequency of responses of whole plants and plant parts to various kinds of ionizing radiations have long been an intensively pursued area of investigation. Sparrow, Binnington and Pond (1958) published the bibliography of the work done on radiation botany during 1896-1955. The various effects of ionizing radiations on plants have been reviewed by Gordon (1957), Gunckel (1957, 1965), Gunckel and Sparrow (1961) and Romani (1966). Comprehensive accounts of radiation effects at atomic, molecular and cellular levels of organization are given in Radiation Research (Silini, 1967).

Sparrow and Forro (1953) and Bacq and Alexander (1961) have discussed in detail the fundamentals of radiobiology. While Sparrow (1960) mentioned the practical applications of ionizing radiations, Ehrenberg (1955) described the mechanisms of action of radiations in seeds. Gray (1954) and Sparrow *et al*. (1961a, b) have discussed the factors affecting the responses of plants to acute and chronic irradiations. Saric (1961) studied the effects of irradiation in relation to the biologic traits of the seed and reported that these have some bearings on the effects of irradiations. Patt (1953) while describing the nature of radiobiological effects discussed problems of radiosensitivity, intermediary radio-chemical events and different factors having some bearing on radiosensitivity of the organism concerned. Variation in the radiosensitivity of plants at interfamily, interspecific and intraspecific levels are reported (Sparrow, 1962, 1964).

Effects of gamma rays on germination of seeds of gymnosperms and angiosperms have been studied by several workers. The higher exposures are usually inhibitory (Bora, 1961; Bowen and Thick, 1961; Saric *et al.*, 1961; De Hondt and Balal, 1966; Johnstone and Klepinger, 1967; Miyazaki, 1968; Bancher *et al.*, 1971; Garg, 1972; Singh and Chowdhury, 1972; Tewari and Chandra, 1975; Rao *et al.*, 1976; Ghosh and Sen, 1978; Venkateswarlu *et al.*, 1978), while lower exposures are sometimes stimulatory (Stotzky *et al.*, 1964; Torne, 1964; Süss, 1966; Vig, 1969; Dnyansagar and Tarar, 1971; Mujeeb, 1974; Torne and Desai, 1975; Mathew and Gaur, 1975; Maherchandani, 1976; Mujeeb and Greig, 1976).

Gunckel and Sparrow (1961) have extensively reviewed the work done on the effect of gamma rays on plant growth and development. From the literature it is evident that one of the most characteristic responses of plants to ionizing radiation is dwarfing owing to destruction or growth inhibition of apical meristems or partial failure of the internodes to elongate. In general, the growth is inversely proportional to the dose rate or total exposure dose of gamma rays administered (Gunckel, 1965). Gamma-ray-induced growth inhibition has been reported in *Lycopersicon* (Sparrow and Singleton, 1953), *Nicotiana* (Meiselman, 1956), cereal seedlings (Dumanovic and Ehrenberg, 1965), *Pinus rigida* (Mergen and Thielges, 1966), wheat, sorghum and radish (Woodstock and Justice, 1967), *Sequoia gigantea* (Taylor, 1968), *Phaseolus vulgaris* (Bajaj *et al.*, 1970), wheat (Hussain and Khan, 1971), *Phaseolus aureus* (Rajput, 1971), *Solanum khasianum* (Chauhan, 1978), *Cyamopsis tetragonoloba* (Misra *et al.*, 1979), gram (Khanna and Maherchandani, 1980), rice (Inoue *et al.*, 1980). Saric *et al.* (1961) working on wheat observed that while lower exposures of gamma rays were stimulative the higher exposures were inhibitory. Sax (1963) while discussing the stimulative effects of ionizing radiation, stated that these stimulative effects are often not reproducible, although in individual tests the difference may be statistically significant. Kahan (1973) demonstrated that very marked increases in vegetative growth may be induced by radiation, without any visible effect on the germination capacity of the irradiated seeds of *Poa pratensis*. Stimulation of growth by gamma rays has been reported in *Phaseolus vulgaris* (Mujeeb and Greig, 1976), *Petunia inflata* (Bapat and Rao, 1976) and *Pisum sativum* (Shamsi and Bajwa, 1978).

Gunckel (1957) reviewed the work done on meristems. Since in the present study only shoot apical meristem was investigated, work pertaining to the shoot apical meristem alone is reviewed. Gunckel and Sparrow (1954) found that in haploid tomato plants unlike the floral apices where the actively dividing cells were highly sensitive, in vegetative apices, compared to their highly vacuolated derivatives, the dividing cells were less influenced . The flattening of irradiated shoot apices has been reported for concorde grape (Pratt, 1959), apple (Pratt *et al.*, 1959; Pratt, 1963), *Prunus avium* (Pratt, 1968), *Pinus rigida* (Bostrack and Sparrow, 1969) and guar (Rai and Singh, 1976). Crockett (1957) reported that the normally low domed shoot apex, with a biseriate tunica of tobacco plants exposed to chronic gamma-irradiation became more elevated, periclinal divisions occurred in first tunica layer and the identity of the second tunica layer was lost. Maximum damage to inner tunica and corpus has been reported in *Lycopersicon esculentum*, *L. pimpinellifolium* (Kuehnert, 1962), apple (Pratt, 1963), apple and pear (Pratt, 1967) and *Linum* (Gunckel, 1965).

Different regions within the shoot apical meristem are differentially sensitive to radiation treatments. Miksche *et al.* (1962) reported that in *Taxus media* the deeper the layer or the region within the meristem proper the less the sensitivity to radiation. Crockett (1968) has also reported the existence of a radiosensitivity gradient in *Coleus blumei* in which the deeper the layer or region the greater the sensitivity to radiations. Pratt *et al.* (1959) stated that in apple shoots exposed to gamma rays the first tunica layer was more resistant than the inner tunica and corpus. The pith rib meristem was, however, most resistant. Clowes and Hall (1966) suggested that the differences in radiosensitivity between the different regions of the meristems are solely due to differences in rates of mitosis of the cells. Foard and Haber (1961) observed that gamma rays caused damage to whole of the shoot apex in wheat and induced inhibition of cell division. Chronic exposures of gamma rays caused maximum damage to apical meristems and axillary meristems of Pinus rigida (Mergen and Thielges, 1966). Pratt (1968) while reviewing the work done on radiation damage in shoot apical meristems, reported that in Prunus avium gamma rays caused maximum damage to scattered cells which could be characterized by darkly stained thickened cell walls and the virtual absence of division. Iqbal (1969) observed abnormal presence of isolated vessels in some of the completely disorganized shoot apices of Capsicum annuum. Iqbal (1969) considers that radiation-induced growth abnormalities are mainly due to cell death and suppression of mitoses at different exposures. The studies of Iqbal (1970) revealed that though resumption of normal growth of shoot apices of Capsicum annuum exposed to low exposures (1-2 kR) can take place by direct recovery of the meristem, no recovery takes place at higher exposures of 4-10 kR. Lapins and Hough (1970) observed that in apple and peach shoot apices the sensitivity to radiation injury decreased from promeristem through peripheral meristem to pith meristem. Graham (1972) made an electron microscopic study of maize shoot apices 24 hours after acute lethal gamma-irradiation and reported that large, dense, intricately formed vacuolar inclusions developed in many interphase cells of the apical meristem. Absence of cytohistological zonation is reported in gamma-irradiated shoot apices of Solanum melongena and Capsicum annuum (Patel and Shah, 1974), Carthamus tinctorius

(Chauhan and Singh, 1975) and guar (Rai and Singh, 1976). Chauhan and Singh (1975) also reported the occurrence of isolated tracheidal cells in apices exposed to 20 kR. Chauhan (1976) stated that the safflower shoot apex has a differential radiosensitivity gradient, the tunica being most radioresistant. Singh *et al.* (1980) studied the effect of gamma rays on shoot apices of *Ricinus communis* and reported that the two-layered tunica seen in unirradiated shoot apices was one-layered in irradiated ones.

Gunckel (1965) stated that in a critical dose range, which varies with the species, in a number of plants flowering may be stimulated e.g., *Nicotiana rustica* (Gunckel and Sparrow, 1954), *Impatiens sultanii* (Gunckel, 1957), *Tradescantia paludosa* (Gunckel *et al.*, 1953). Both early and delayed flowering as a response to gamma-irradiation are known (Sparrow and Singleton, 1953; Sax, 1955; Spencer, 1955; Bowen *et al.*, 1962; Seetharam and Srinivasachar, 1976; Maltseva, 1978; Selenina and Stepanenko, 1979; Katyayani *et al.*, 1980; Tarar and Dnyansagar, 1980). However, Amer and Hakeem (1964) reported that in *Lupinus termis* flowering time was not influenced by gamma-irradiation. Pre-sowing gamma-irradiation of seeds resulted in early maturation of fruits and accelerated and enhanced inflorescence development in tomato (Kahan *et al.*, 1972; Kahan, 1974) and pepper (Maltseva, 1978). Low exposures of gamma rays induce increased flower formation in *Pisum sativum* (Shamsi and Bajwa, 1978).

Radiations are known to influence anther and pollen development, female gametophyte and embryo development. Since these aspects do not constitute part of this investigation, literature on these aspects are not reviewed here.

Pollen grains are suitable material for radiobotanical investigations since they afford a relatively simple haploid radiation target and can withstand an impressive array of experimental conditions. The literature pertaining to pollen radiobotany has been extensively reviewed by Brewbaker and Emery (1962) and Brewbaker et al. (1965). Pfahler (1967, 1971, 1973) conducted detailed experiments on the effect of gamma and u.v. irradiation on maize pollen. Gamma-irradiation upto 5 kR though did not alter in vitro germination, tube length and fertilization ability of the pollen, kernel set was drastically reduced (Pfahler, 1967). Pfahler (1971) studied the effect of high exposures of gamma rays (upto 120 kR) on in vitro responses of pollen and observed a linear decrease in germination percentage with increasing exposure. Gresti et al. (1977) have studied the ultrastructural aspects of pollen tube growth following gamma irradiation in Lycopersicon peruvianum. Van der Donk et al. (1978) reported that X-irradiation of pollen in Douglas-fir resulted in interference with the main regulatory mechanism of pollen tube growth, which is de- and re-masking of mainly pre-synthesized m RNA.

The effects of ionizing radiations on the yield of various agricultural crops have been studied. Yamakawa and

Sparrow (1965) observed reduction in viable seed set by chronic gamma-irradiation in twentyone cultivated plants. Reduced crop yields following irradiation are reported in literature (Davies, 1968, 1973; Kahan, 1969; Ashraf _et_ _al_., 1975; Fautrier, 1976; Iqbal, 1980). Compared to stem length, yield showed a greater degree of sensitivity to gamma-irradiation in _Glycine_ _max_ (Killion _et_ _al_., 1971). Katiyar and Roy (1973) reported that though yield was increased to the extent of 60, 30 and 15% in 30, 40 and 20 kR irradiated plants of _Citrullus_ _lanatus_ respectively, the seed fertility decreased with increased exposure of radiation.

Enhanced yield as a radiation response is reported in oil mustard, peanut, peas, sesame, barley, oats and wheat (Sparrow and Konzak, 1958), Corn (Berezina _et_ _al_., 1962), Safflower (Singh, 1974), pea (Shamsi and Bajwa, 1978) and broad bean (Shamsi and Sofajy, 1980). Ibraginov _et_ _al_. (1961) observed that gamma-irradiation of cotton seed resulted in an increase in the number of bolls per plant and the oil content in the seed.

Nicotiana plants raised from gamma-irradiated seeds have a higher nicotine content at the ripening stage, compared to control (Kuzin _et_ _al_., 1963). In gamma-irradiated _Mentha_ _arvensis_ oil percentage and menthol content did not differ significantly from the progenies of the untreated stolons (Mital _et_ _al_., 1972). Kaul _et_ _al_. (1973) reported that in _Datura_ [illegible] 10 kR of gamma rays caused a remarkable increase of total alkaloids. Tarar and Dnyansagar (1977) observed that caffeine content increased in Turnera ulmifolia plants raised from seeds treated with lower exposures of gamma rays or concentrations of EMS. Sadowska (1979) reported a significant increase in the percentage of essential oil in the leaves of peppermint as a result of irradiation. Selenina and Stepanenko (1979) noted an increase in ester oil content in the inflorescences of Matricaria recutita plants raised from gamma-irradiated seeds.

Growth hormones:

Phytohormones have a profound influence on the growth processes in plants (Hall, 1973; Jones, 1973; Wareing and Phillips, 1978) and are also known to modify radiation responses in plants (Klein and Klein, 1971). Since using growth hormones an attempt is made, in the present study, to modulate the radiation effects on the expression of heterostyly, pollen germination and pollen tube growth, the literature on these aspects only is reviewed here.

Johri et al. (1977) stated that the response of pollen grains to exogenous growth hormones is not well understood. There are a few reports of stimulation of germination and tube elongation by auxins, gibberellins and cytokinins (Chandler, 1957; Konar, 1958; Bose, 1959; Raghavan and Baruah, 1956, 1959). According to Sondheimer and Linskens (1974), however, IAA, gibberellins, zeatin and abscisic acid do not affect in vitro germination and tube growth of Petunia hybrida. McLeod (1975) observed that tomato pollen was almost completely insensitive to plant growth substances (including auxin, gibberellins and abscisic acid). The application of IAA at a low level (1 ppm or even less) at 1, 2, 3 hour after culture failed to influence pollen germination and the rate of tube elongation,but at higher levels (100 ppm) it suppressed germination. However, when high concentrations of IAA were added 3 hours after culture there was a pronounced stimulatory effect on tube growth. At higher concentrations several other growth inhibitors (maleic hydrazide, iodoacetate, transcinnamic acid and abscisic acid) also inhibited germination to various extent, but promoted tube growth. This led McLeod (1975) to suggest that germination and tube elongation are two distinct processes differing in their sensitivity to different chemicals such as maleic hydrazide, transcinnamic acid, iodoacetate and abscisic acid. Dickinson (1978) observed that neither early pollen tube growth nor percent germination was stimulated by the hormones (IAA and GA) or was there any effect by AMO-1618, an inhibitor of gibberllin biosynthesis. Addition of GA in the growth medium markedly inhibited germination and tube growth in Lycopersicon esculentum (Dhingra and Varghese, 1976). According to Prasad (1976) increasing concentration of GA, IAA and IBA increased pollen tube length of Eriobotrya japonica. Chhabra and Malik (1976) opined that auxin played a significant role in hastening germination and eliciting tube length over control in groundnut. They are of the view that in Arachis hypogea auxin seems to act at the germination stage while gibberllin at the early and ethylene at the pollen tube stages, respectively. Yadav (1980) studied the effect of growth regulators on pollen germination and pollen tube growth in Cassia tora and C. obtusifolia and showed that IAA failed to enhance both germination and tube elongation. GA_3 could improve germination a little at 100 mg/l in C. tora and at 20 mg/l in C. obtusifolia, the pollen tube elongation remained under control value, however.

Wakhloo (1975b) has shown that while GA_3 treatments enhance production of female sterile flowers in Solanum sisymbrifolium plants low in potassium content, kinetin application enhances development of female fertile flowers. Reddy and Bahadur (1977) consider that the cause of female sterility of short styled flowers in S. surattense is probably hormonal and sporophytically determined.

Modulation of radiation responses:

The responses to ionizing radiation 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 treatment of gibberellic acid modulates the growth responses of radiation-stunted maize seedlings (Gaur and Notani, 1960), wheat seedlings (Haber and Luipold, 1960;

El-Keredy et al., 1977) and pinto bean plants (Lockhart, 1961). Mathur (1965) using GA could reverse r-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 soybean plants. Silveira and Hell (1977) stated that post-irradiation GA_3 treatments show modulatory effects reducing the severity of radiation-induced growth inhibition in Phaseolus vulgaris plantlets.

Holmsen et al. (1964) using auxin reported reversal of radiation inhibited geotropic response in corn and pea seedlings. Cerkovnikov et al. (1965) while studying action of some biocatalysts on soybeans, unirradiated and irradiated with X rays, found that 2,4-D rendered partial protection against the inhibiting action of radiation. Romani (1966) suggested that auxins might have a significant role in correcting inhibitory effects of gamma irradiation. Abrol et al. (1969) observed that the decreased α-amylase activity induced by gamma rays in wheat could be reversed by pre-sowing soaking in IAA solution. When rice seeds exposed to 5 kR of gamma rays were supplied IAA (50 and 100 ppm) exogenously there was a significant recovery in growth (Bhattacharya and Rao, 1978).

Araratyan et al. (1975) studied the radioprotective effects of kinetin on chromosome aberrations induced by irradiation of Crepis capillaris seeds. They found that kinetin stimulates the onset of stage S for a certain proportion of cells and thus at the moment of irradiation the cell population is in a asynchronous state. Mikhailov *et al.* (1978) found that the post-irradiation soaking of pea seeds in kinetin solution removes partially the inhibitory effect of X-ray-irradiation on the growth and development of seedling axial organs as well as the growth and development of lateral roots.

Solanums

(i) *Solanum khasianum*:

Solanum khasianum is widely distributed in India (Kaul and Zutshi, 1977; Sharma and Varghese, 1980). Deb (1975) considers that *S. myriacanthum* Dunal is the species having various synonyms (*S. reflexum* Schrank, *S. platanifolium* Hooker, *S. viarum* Dunal, *S. khasianum* Clarke var. *chatterjeeanum* Sengupta). According to Babu and Hepper (1979) *S. khasianum* Clarke is considered as conspecific with *S. myriacanthum* Dunal which is the acceptable name for this species. Thus the nomenclature of this species is controversial.

S. khasianum is an erect or somewhat prostrate, branched undershrub or shrub upto 1.5 to 1.8 m high. Spines are present on the stems, petioles, leaves and pedicels of flowers. Leaves are ovate having triangular lobes, hirsute and spines are present on both the surfaces. Flowers are white, in lateral 1-4 flowered racemes. Berries are pale yellow with green streaks when immature and bright yellow on maturity. Seeds are smooth, brown and compressed.

The somatic chromosome number of S. khasianum is 24 (Bezbaruah and Bezbaruah, 1963; Mitra, 1966, 1967; Chennaveeraiah and Krishnappa, 1965; Gill, 1972; Dnyansagar, 1976b). Krishnappa (1968) and Mehra and Gill (1968) reported the meiotic chromosome number as n = 12. The presence of B-chromosomes in this species were reported by Dnyansagar and Dhanraj (1972).

Studies on the germination of S. khasianum seeds are very limited. Patil (1967) studied the effect of gamma rays on germination behaviour and seedling growth. Though the rate of germination was delayed, no correlation was obtained between germination percentage and dosage. Bhatt (1972) irradiated dry seeds with 5 to 35 kR exposures of gamma rays and reported 20 kR exposure as the LD_{50} for seed germination; exposures above 30 kR were lethal. Chauhan (1978a), however, observed that S. khasianum seeds exposed to 25 kR of gamma rays, failed to germinate. Chauhan (1978b) and Pingle and Dnyansagar (1979) have reported the erratic germination of seeds. According to Chatterjee (1977) March-April sowing of the seeds resulted in 40% germination and in August-September sowing only 30% seeds germinate. Chauhan (1978b) observed that while lower concentrations of ethrel (500 and 750 ppm) stimulated seed germination, the higher concentrations (1000 and 1250 ppm) inhibited and delayed germination. Laha and Basu (1980) reported that gibberellic acid replaced the light requirements of seeds suggesting that the seeds of S. khasianum are positively photoblastic in nature. Amin Uddin and Chaturvedi (1980) have reported the presence of abscisic acid in the berries of S. khasianum and consider that the erratic and low percentage of germination may be due to the presence of abscisic acid in the berries. Mitra and Kushari (1981) observed that while low concentrations of 2,4-D were effective in breaking dormancy initially, higher concentrations increased the percentage of germination in late period of storage.

According to Chakraverty and Basu (1973) foliar application of gibberellic acid (GA_3) resulted in an increase in plant height with a considerable reduction in the number and length of spines on both the surfaces of the leaf, petiole and stem as compared to untreated control. The leaves of treated plants were soft in texture, less lobed, slightly pale in colour and almost spineless. They consider that the suppression or reduction in the formation and development of spines are related to the rapid elongation of plants and expression of apical dominance as a result of GA_3 treatment. Datta et al. (1973) reported that phloridzin, a chalcone derivative, at the highest concentration (1000 mg/l) stimulated plant height, leaf number and yield of berries in S. khasianum. Chauhan (1978a) studied the gamma-ray-induced variation in the development of S. khasianum. He observed that gamma-ray exposures inhibited the height of the plants, number of leaves produced per plant as also number of branches per plant. Further gamma rays affected spine number, spine intensity, and spine size of the spines present on the leaves.

In S. khasianum flowering initiates within 65 to 80 days of sowing and start blossoming in the early hours of morning (Chandra et al., 1970). Kaul and Zutshi (1977) reported that flowering occurs about 55-65 days after transplanting. Pal and Singh (1979) studied the blossom biology of S. khasianum and reported that initiation of flowering occurs within 65 to 80 days of seed sowing. The development of flowers after the emergence of buds required 20 and 25 days and blooming period was 130 and 150 days in JRL-28 and JRL-12, respectively. The duration of flowering was 100 days.

Pollen morphological studies in this species were made by Basak (1967) and Sharma (1974). The media requirements for the germination of pollen grains of S. khasianum were determined by Ravindran and Chauhan (1980).

Murthy and Abraham (1975a) observed heterostyly in both the normal wild and the curved mutant varieties of S. khasianum. The length of the style varied from less than a mm to as much as 15 mm. The frequency of occurrence of these two types of flowers varied with the age of the plant. Crossing experiments revealed that the most successful crosses were those where there was a long style. Fruit set was nearly 90% in such cases. However, no fruit set was observed in crosses with short styles.

Mohan (1970) studied the embryological aspects of S. khasianum. The ovules of this species are anacampylotropous, unitegmic and tenuinucellate. One, rarely two archesporial cells directly function as megaspore mother cell or cells. Usually linear, but in some cases a 'T'-shaped tetrad of megaspores is found. The chalazal megaspore functions. The development of embryo sac is of polygonum type. The synergids are narrow and the antipodals are ephemeral. Polar nuclei move upward and fuse to form the secondary nucleus in the upper half of the embryo sac. Before male nucleus is able to fuse completely with primary endosperm nucleus, it divides. Pollen tubes are present even at the second or third division of zygote.

Chauhan _et_ _al_. (1975b) studied the association between yield of fruits and its components and reported that significant positive correlation exists between number of fruits, weight of fruits, size of fruits and yield per plant; the highest being between number of fruits per plant and yield of fruits.

Kammathy _et_ _al_. (1971) described the pharmacognostic aspects of the berries. The berries are nearly spherical with a diameter of 2.3 to 3.2 cm, deep green with white variegation when young and turning bright yellow on full maturity, smooth and glossy in texture. The placentation is axile with innumerable seeds embedded in soft pericarp. Seeds are compressed, flat with the outline circular or nearly so and covered with a mucilage layer. Anatomical studies of the fruit have shown the presence of a distinct cuticle with 2 or 3 layered, small sized epidermal cells with thick walls. There are two inner layers of cells containing either prismatic or rectangular crystals. Below the epidermal layer there are 2 or 3 layers of collenchyma cells. The rest of the pericarp and placenta are made up of multilayered parenchymatous cells composed of outer closely packed and inner loosely packed cells. Vascular tissue is scattered among parenchyma cells in the inner region of the pericarp and placenta. Vessels are with spiral thickening. Seed is composed of a thick walled epidermis followed by crushed cells of the inner integument and perisperm cells. The embryo is narrow, curved and lies enclosed by the endosperm cells. The cells of both the embryo and the endosperm are filled with granular contents.

Macroscopic examination of powdered berries give a greenish brown colouration with pungent smell, slightly bitter in taste and highly mucilaginous. Microscopical examination reveal multicellular pointed trichomes and uni- or multicellular trichomes with stomata. Microchemical tests reveal the presence of glycoalkaloid and calcium oxalate crystals in the inner epidermal cells of the pericarp.

Conflicting reports have appeared on the alkaloidal content of *S. khasianum*. The berries of *S. khasianum* var. *chatterjeeanum* is the richest source of solasodine with an yield of 5.4% on a dry weight basis (Maiti *et al.*, 1964). Other workers (Chaudhuri and Rao, 1964; Chaudhuri and Hazarika, 1966), however, obtained far too low values of solasodine. Maiti *et al.* (1965) made a comparative assay of berries collected from different localities in India and reported that the berries collected from Niligiris contained the highest amount of solasodine. Saini (1966) analysed the fruits of same physiological age at about weekly intervals right from their early development and reported a progressive accumulation of glycoalkaloid. The concentration of glycoalkaloid reached a maximum (7.4%) when the fruits were about 55-60 days old. This stage of development appears to coincide with the fruit colour changing from green to yellow. Chandra _et_ _al_. (1970) reported that solasodine content ranged from 0.10 to 0.87% upto 5th week of fruit development. In the 6th week with change of colour the increase in solasodine content was considerably higher ranging between 1.55 to 1.89% which continued upto 10th week. Khanna and Murthy (1972) found that alkaloid was maximum when berries were deep yellow. The berries harvested from older plants have higher alkaloid. They further suggested that though the interaction of genetic make up, plant maturity and stage of harvest have an important bearing on the alkaloid content of the berries, temperature possibly does not influence this accumulation. Sharma _et_ _al_. (1979) reported that maximum content of solasodine was 80 days after fertilization when the colour of the fruits was turning yellow. Decrease in solasodine content was observed upto the full ripening of the berries (complete yellow). Varghese _et_ _al_. (1979) stated that solasodine which is synthesized in the leaves and berry walls is continuously accumulated in the fruit pulp. The maximum amount of solasodine is obtained at the pale yellow stage of the berries.

Analysis of various parts of the fruit indicates that the fleshy cover and washed seeds do not contain any alkaloid, which is concentrated in a mucilage layer all around the seeds (Saini, 1966). Saini and Biswas (1967) further enumerated that this mucilaginous layer develops as a result of disintegration of the outermost layer of developing seeds and appears to be more connected with the development of the seed itself than the remaining part of the fruit. Hence the total alkaloid content may thus be directly correlated to the number of seeds in each fruit. This was further substantiated by the analysis of Bakshi and Hamied (1971) who obtained 1.0 and 1.6% solasodine in the seed and mucilage of ripe berries, respectively. This was, however, disputed by Janaki Ammal and Bhatt (1971) as they got entirely different results in diploid and tetraploid plants. According to them, the fruits obtained from diploid plants though had 200 to 300 seeds, the glycoalkaloid content was appreciably less than that of fruits of tetraploid plants containing 2-5 seeds. Murthy (1976) studied the alkaloid distribution in _S. khasianum_ and observed a correlation between seed number and fruit size, but no correlation was found between the glycoalkaloid content and fruit size. According to him the mucilage secreting cells reponsible for the glycoalkaloid are not confined only to the seed coat but are also present in other parts of the fruit.

Datta _et al._ (1973) found that lowest concentration of phloridzin (1mg/l) increased the yield of solasodine.

Chaudhuri and Chatterjee (1979a) observed that sucrose feeding generally increased the formation of solasodine and the effect was dependent on the age of the fruits. Chaudhuri and Chatterjee (1979b) also found that altitude has an influence on solasodine content of fruits; the effect being inhibitory and seasonal in character. Further, varying amounts of GA_3 - feeding increased flowering and fruiting as also solasodine content.

Several attempts were made to cross *S. khasianum* with 22 other species available but no success was achieved (Kaul and Zutshi, 1977). Interspecific hybridization even with species which show close karyomorphological relation with it did not succeed *(Zutshi, 1968). Sarkar *et al.* (1977) made an estimate of genetic divergence between some solanaceous species and reported that *S. khasianum* was more closely placed to *S. nigrum*. They thereby suggested that to improve plants of *S. khasianum* some interspecific crosses should be made either with *S. nigrum* or via this species.

Tetraploidy was induced by treating young seedlings with colchicine, before plumule development (Janaki Ammal and Bhatt, 1971). Tetraploids showed delayed flowering and fruiting but possessed fewer spines than the diploids. The fruits in tetraploids were smaller in size and weight, but contained larger seeds. An interesting feature noticed in these plants was the presence of 3-5 fruits at some nodes instead of usual single fruit in diploids, indicating the development of more than one hermaphrodite flower in tetraploids. Janaki Ammal

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* Quoted from Kaul and Zutshi (1977)

and Bhatt (1971) pointed out this feature to be an added advantage in commercial cultivation.

Bhatt (1972) irradiated dry seeds of *S. khasianum* with 5-35 kR of gamma rays. One of the M_2 progenies isolated from 10 kR treatment showed thick curved and blunt spines with a 56% reduction in spine length. The mutant plants were taller than control. The increase in yield was due to larger size of fruits in the mutants. The glycoalkaloid content also showed an improvement over control. Bhatt (1972, 1977) obtained almost spineless plants after treatment with gamma rays and colchicine treatment respectively and oberved that reduction in spine number and size was associated with sterility. Murthy and Abraham (1975b) treated *S. khasianum* seeds with 20 and 30 kR of gamma rays. Some of the highly vigorous R_2 plants were treated with 0.01 and 0.02% Nitrosomethylurea (NMU). A M_3 progeny from the former treatment produced some plants which were almost completely spineless, fully fertile and produced fruits profusely. The stems, younger leaves and floral parts were all devoid of spines. Chauhan *et al.* (1976) have shown an increase of 166.6% of berry yield over control in M_2 generation of 20 kR irradiated plants. Chauhan *et al.* (1975a) also found that the radiation treatment did not change solasodine content appreciably vis-a-vis control. Kaul and Zutshi (1974) evolved several high yielding strains by exposing dry seeds to various dosages of gamma rays (7.5-20 kR) and chemical mutagens like EMS (0.05 and 0.15%), ethyleneimine (0.025%) and nitrosoguanidine (0.5 mM).

They isolated a good number of mutants showing significant vegetative growth, fruit setting, less frequency of spines and resistance to diseases. One mutant (RRL - 20 - 2) was found to be very promising in having vigorous vegetative growth, 3 to 4 fruits per node, reduced number of spines on leaves with almost spineless stems (Kaul and Zutshi, 1977). The yield of berries in mutant was found to be 5.51 tonnes per hectare with solasodine content of 3.0% on dry weight basis resulting in net yields of 41.25 kg of solasodine per hectare as against control where 6.12 tonnes of berries with 1.2% solasodine yielded about 18.15 kg of solasodine per hectare (Kaul and Zutshi, 1977).

Hazarika *et al.* (1978) selected a superior strain JRL-30 after extensive field trials. It produced 6.14 tonnes of fresh berries per hectare with 3.28% solasodine on dry weight basis amounting to 40.34 kg of calculated solasodine per hectare. This selection had less percentage of mortality (4.7 to 9.7%) in field with 73% seed germination.

Gandhi (1978) carried out agronomic evaluation of *S. khasianum* and reported a significant variation in the number of branches and the number of berries per plant. The solasodine content also ranges from 0.6 to 2.2%. Singh *et al.* (1978) have studied the genetic variability for gluco-alkaloid content in *S. khasianum* and suggested that production of gluco-alkaloids is a quantitative character controlled by genetic mechanisms.

S. khasianum plants are fairly hardy and can thrive well under diverse climatic conditions. However, they cannot stand too much of desiccation or water logging. Such conditions not only affect very adversely their normal growth but result in scorching of the leaves with gradual yellowing and ultimate wilting of the plant (Chakraverty and Raychaudhuri, 1974).

The plants are generally propagated by seed but vegetative propagation by stem cuttings are also successful (Kushari and Chatterjee, 1976). The seeds are either sown in nursery or seed beds to raise seedlings for transplantation or broadcast for direct sowing. Singh et al. (1972) indicated that direct sowing of seeds in the field gives a significantly better yield of fruits as compared to sowing by transplantation. The optimum period for seed sowing was October. Hazarika and Bora (1976) found that a spacing of 90 cm x 90 cm gave maximum yield of berries.

The time of sowing (March, June and October) plays a significant role in the growth, development and ultimate yield of fruits without much affecting the glycoalkaloid contents (Saini and Biswas, 1967). According to Chandra et al. (1970) plants transplanted in August exhibit good growth with stout branching.

Fertilization in newly introduced areas is seldom essential, but the plants show great agronomic promise and responds well to fertilizer applications (Chakraverty and Raychaudhuri, 1974). N, P and K have been found to increase the growth of plants and fruiting. Excess of N fertilizers reduce the fruit yield and the plants become susceptible to wilting. Applications of P_{40}, K_{40} and N_{40} and N_{60} per hectare have been found to produce the highest number of fruits. In case of pot culture 1 kg of farm yard manure supplemented with monthly applications of 2 g each of ammonium sulphate, potassium sulphate, and superphosphate produce good growth of plants (Saini and Biswas, 1967). Chaudhury *et al*. (1979) reported increased yield of berries due to nitrogen application. This also resulted in a marginal increase in solasodine content.

The plants are generally cultivated as annuals and complete the maximum fruiting within 5 to 6 months after sowing subsequent to which the flush of flowers and fruit setting gradually declines. Under suitable cultural practices and favourable climatic conditions the plants grow as perennials. For fruit production, maintenance of continuous supply to the industries and to get maximum benefit it is advisable to raise at least two crops of this species per year in the same land. However, perennial populations may also be maintained for seed collection particularly when some high yielding strains are isolated (Chakraverty and Raychaudhuri, 1974).

Khanna *et al*. (1976) studied the solasodine content in tissues grown in static cultures and reported the maximum amount of solasodine (0.025%) in 8 weeks old tissue. Kokate and Radwan (1978) established the callus cultures of *S. khasianum* on MS and B_5 media and found that callus cultures at the beginning of organ differentiation, were much richer in steroidal glycoalkaloids (5.2% of tissue dry weight), compared to seeds (0.34%). Amin Uddin (1978) and Amin Uddin and Chaturvedi (1979) reported that the callus cultures of S. khasianum derived from whole seedlings were capable of synthesizing solasodine upto 0.067%. Chaturvedi et al. (1979) compared to alkaloid biosynthetic potential of seed-callus with seedling-callus. Compared to seed-callus, seedling-callus synthesized less alkaloid. Chaturvedi and Sinha (1977, 1979) have reported the mass clonal propagation of S. khasianum through tissue culture techniques. Bhatt et al. (1979) observed that leaf explants of S. khasianum did not regenerate on any tested media in the light, but did so when cultured in darkness.

Thakur and Sastry (1971) and Rangaraju and Chenulu (1974) described the host range, symptomatology and properties of the virus causing mosaic disease of S. khasianum. Verma et al. (1972) and Rangaraju and Chenulu (1974) designated the virus isolated from S. khasianum as SKMV. Rangaraju and Chenulu (1975) reported a significant reduction in growth and alkaloid content of virus infected S. khasianum plants. Chlorotic stunt disease and its association with root-knot nematode Meloidogyne javanica has been reported by Zaidi et al. (1978). Root-knot and wilt diseases caused by Meloidogyne incognita could be controlled by carbofuran application at the rate of 25 kg per hectare at an interval of three weeks (Mazumdar et al., 1977). Ismail et al. (1979) reported the influence of root-knot nematode and tobacco mosaic virus on the growth and carbohydrate content of this species. Krishna Prasad _et al._ (1980) observed increased wilting of _S. khasianum_ plants treated with the nematode _Meloidogyne incognita_ and the fungus _Corticium rolfsii_ thus indicating a high degree of synergism between the two pathogens. The wilt disease of _S. khasianum_ is caused by _Fusarium oxysporum_ and the incidence of disease in Jorhat conditions, can be controlled by direct sowing of seeds rather than transplanting (Bordoloi _et al._, 1971). Siddaramaiah _et al._ (1979) reported that the fungicides Vitavax and Plantavax were effective in controlling root wilt of _S. khasianum_. Besides these the other diseases of this species are sclerotial rot (Upadhyay and Bordoloi, 1977), leaf blight (Gupta _et al._, 1979) bacterial wilt (Ram Kishun _et al._, 1980), and _Alternaria_ fruit rot (Hiremath _et al._, 1980).

(ii) _Solanum indicum_:

The importance of _Solanum indicum_ as an alternate source of starting material for the synthesis of steroidal hormones was revealed by the alkaloid screening study of Chopra and Handa* (1963). _S. indicum_ fruits contain 4.8% crude glycoalkaloid and 1.8% of the alkaloid. Rathore _et al._ (1978) reported the presence of steroids and steroidal alkaloids in the stems, leaves, roots, flowers, green and ripe berries of _S. indicum_ and identified them as diosgenin, β-sitosterol, lanosterol, solasonine and solamargine. The solasodine content was 0.347% in green berries.

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* Quoted from Chopra and Kapoor (1968)

S. indicum L. is an erect, branched, undershrub upto one metre high. The stem is armed with spines and stellate tomentose hairs. Leaf is shallowly lobed, ovate, oblong, somewhat oblique. Inflorescence is cymose, few to many flowered; pedicel 1-5 cm long, smooth or prickly; calyx sometimes prickly; corolla blue, slightly stellately hairy 1-1.5 cm long. Berries are globose 1 cm across, green when immature and bright red on maturity. Seeds are pale yellowish, flattened.

Viswanathan (1975) reported that *S. indicum* which is a diploid has 2n chromosome number = 24. He induced tetraploidy in this species by colchicine treatment.

Very few studies have been made on the growth and development of *S. indicum*. Kharbteng and Chauhan (1979) studied the effect of ethrel on *S. indicum* which revealed a reduction in the number of stamens and absence of pistil in some flowers.

The media requirements for pollen germination of *S. indicum* were studied by Ravindran and Chauhan (1980).

The response of excised anthers of *S. indicum* in vitro was studied by David and Chinchanikar (1980).

CHAPTER III

SEED GERMINATION AND SEEDLING SURVIVAL

INTRODUCTION

Several solasodine yielding species of *Solanum* display poor, erratic germination and uneven, slow seedling emergence (Sudiatso and Wilson, 1974; Chatterjee, 1977; Chauhan, 1978b; Pingle and Dnyansagar, 1979). This poses a serious problem in large scale commercial cultivation programmes for the production of steroidal glycoalkaloid solasodine, as transplanting of seedlings raised from seeds is necessary. Various chemical (Spicer and Dionne, 1961; Singh, 1974; Porter and Gilmore, 1976; Chauhan, 1978b; Basu and Chakraverty, 1979; Pingle and Dnyansagar, 1979; Mitra and Kushari, 1980, 1981) and physical agents (Singh *et al.*, 1974; Mathew and Gaur, 1975; Torne and Desai, 1975; Maherchandani, 1976) are used for improving seed germination in problem species. Gamma rays are known to influence seed germination in various crop plants. While lower exposures are sometimes stimulatory (Stotzky *et al.*, 1964; Torne, 1964; Sparrow, 1966; Süss, 1966; Tavčar, 1966; Dnyansagar and Tarar, 1971; Mathew and Gaur, 1975; Torne and Desai, 1975; Maherchandani, 1976), the higher exposures are always inhibitory (Matsuo and Onozawa, 1961; Saric *et al.*, 1961; Johnstone and Klepinger, 1967; Torne and Desai, 1975). Prasad and Godward (1975) consider that relationship between germination and survival of seedlings is of interest in breeding programmes because, although, the germination percentage may not be reduced with high doses, survival may be reduced/negligible or there may be no survival (Fujii and Matsumara, 1958; Micke, 1961). Gamma rays are being used for the improvement of the solasodine yielding Solanum species (Bhatt, 1972; Chauhan *et al.*, 1975a, 1976). But no information is available as to how seed germination and seedling survival is affected by gamma rays in *S. khasianum* and *S. indicum*. An attempt was therefore made to study these aspects in these two species, since this will help in understanding the radiobiology of these species.

MATERIALS AND METHODS

In the present study, seeds used for irradiation were always collected from a single plant of *S. khasianum* and *S. indicum* grown secluded in the previous year. Seeds of *S. khasianum* (moisture content 14.47%) and *S. indicum* (moisture content 14.17%) were sent to the Bhabha Atomic Research Centre, Trombay, Bombay for irradiation purposes. The seeds were given 2.5, 5, 10, 15, 20, 25 and 30 kR exposures of gamma rays emitted from Co^{60} source at the rate of 4.1 kR/minute. The irradiated seeds were flown to Shillong and sown in pots filled with 1:1 mixture of soil and farmyard manure. Per treatment 250 seeds were sown at the rate of 50 seeds per pot. The pots were watered regularly to maintain sufficient moisture for germination. Data on seed germination were recorded regularly upto 63 days after sowing subsequent to which no seedling emergence occurred. The parameters taken into consideration were the days taken for:
(a) commencement of germination (b) achieving 50% germination
(c) completion of germination and final germination percentage.

Seedling survival was scored at the end of 75 days after sowing.

RESULTS

S. khasianum :

Unirradiated *S. khasianum* seeds, start germinating on the 22nd day after sowing, achieve 50% germination in 46 days and complete germination in 60 days. The final germination percentage towards the end of germination was 60 (Table 1). A scrutiny of radiation effect on seed germination revealed that irradiated seeds required almost the same time as control to complete germination. However, the rate of germination in the seeds given low exposures of gamma rays (2.5 - 10 kR) was faster compared to unirradiated control since these seeds achieved 50% germination in lesser time (Table 1). Further, compared to control the final germination percentage was significantly stimulated* by 2.5 kR - 15 kR exposure of gamma rays (Table 1, Fig. 1). The increased seed germination in the seeds given 20 - 30 kR exposures did not differ significantly from control (Table 1).

In unirradiated *S. khasianum* 95.93% seedlings survived. The survival in the irradiated seedlings was though enhanced by low exposures (2.5 - 10 kR), the differences were not significant (Table 2). The higher exposures caused significant inhibition (Table 2, Fig. 1). The maximum injury of 19.95% to seedling survival was induced by 30 kR (Fig. 1).

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* Percent inhibition/stimulation = $\frac{\text{Control - irradiated}}{\text{Control}}$ x100

Table 1: Effect of gamma rays on seed germination in <u>Solanum khasianum</u> and <u>S. indicum</u>.

Treatment	Days taken for germination to commence		Days taken for 50% germination		Days taken to complete germination		Final germination percentage	
	1	2	1	2	1	2	1	2
CONTROL	22	16	46	20	60	46	60.0 ± 7.05	96.8 ± 0.79
2.5 kR	22	16	28	22	62	54	79.6* ± 3.43	90.8 ± 1.02
5.0 kR	22	16	36	22	62	48	80.4* ± 6.12	89.2 ± 1.62
10.0 kR	22	18	24	22	62	50	90.4* ± 2.48	87.2* ± 1.02
15.0 kR	22	18	46	24	62	56	74.8* ± 4.23	81.2* ± 0.80
20.0 kR	24	20	52	22	62	60	61.6 ± 3.06	77.6* ± 2.64
25.0 kR	24	20	46	24	60	58	63.2 ± 4.59	72.0* ± 7.28
30.0 kR	24	22	46	-	60	36	60.8 ± 4.08	28.0* ± 1.67
L.S.D.(p=0.05)							13.0	8.56

1 <u>S. khasianum</u>
2 <u>S. indicum</u>
± S.E.
* Significantly different from control (p=0.05)

Table 2: Effect of gamma rays on seedling survival in *Solanum khasianum* and *S. indicum*.

Treatment	Total number of seedlings		Number of surviving seedlings		percent survival	
	1	2	1	2	1	2
CONTROL	150	242	143	220	95.33	90.90
2.5 kR	199	227	194	214	97.48	94.27
5.0 kR	201	223	197	202	98.00	90.58
10.0 kR	226	218	224	193	99.11	88.53
15.0 kR	187	203	175	183	93.58	90.14
20.0 kR	154	194	147	147	95.45	75.77*
25.0 kR	158	180	133	61	84.17*	33.88*
30.0 kR	152	70	116	0	76.31*	0.0*
L.S.D.(p=0.05)					8.23	6.94

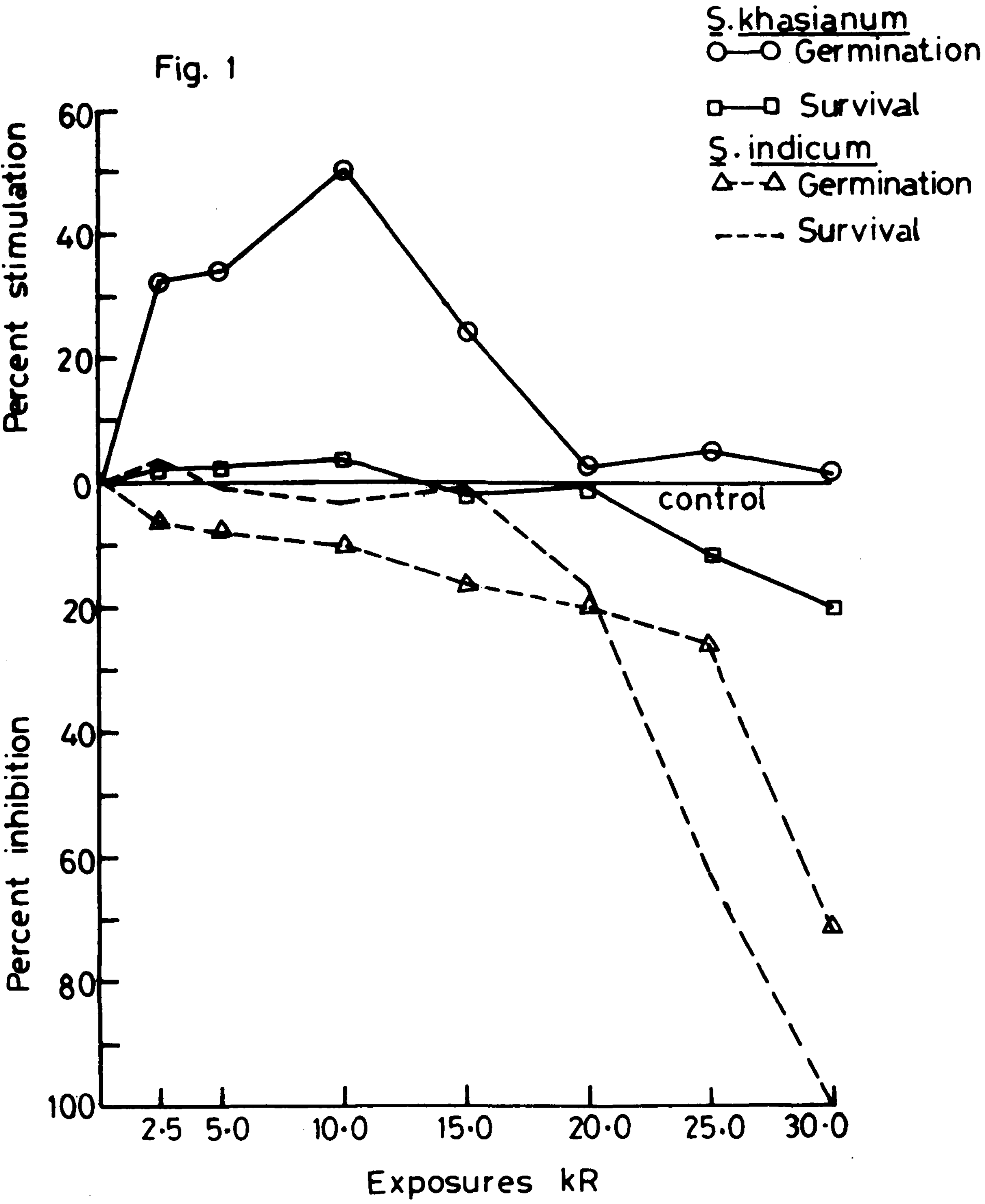
1 *S. khasianum*

2 *S. indicum*

* Significantly different from control (p=0.05)

Fig. 1: Gamma-ray induced percent stimulation/injury for seed germination and seedling survival of *S. khasianum* and *S. indicum*.

Fig. 1


S. khasianum
Germination
Survival
S. indicum
Germination
Survival
Percent stimulation
Percent inhibition
60
40
20
0
20
40
60
80
100
control
2·5
5·0
10·0
15·0
20·0
25·0
30·0
Exposures kR

S. indicum :

S. indicum seeds start germinating on the 16th day after sowing, achieve 50% germination in 20 days and require 46 days to complete germination. The final germination percentage in the unirradiated control was 96.8 (Table 1). Irradiation of seeds delayed germination, adversely affected the rate of germination and reduced the final percentage of germination (Table 1, Fig. 1). Although the effects on the time taken to complete 50% germination and the number of days required for germination to be over were not exposure dependent, the inhibition of seed germination increased with increasing exposure. The inhibitory effect was significant, however, only from 10 kR onwards (Table 1).

In unirradiated S. indicum 90.9% seedlings survived (Table 2). Amongst the irradiated material though survival in the seedlings given 2.5 kR of gamma rays was slightly more compared to control the difference was statistically insignificant. Exposures of 5 kR and above reduced seedling survival but the differences were significant only in 20, 25 and 30 kR exposed seeds (Table 2). The LD_{50} for seedling survival was between 20 and 25 kR and 30 kR proved lethal (Table 2, Fig. 1).

DISCUSSION

A comparison of gamma-ray effects on seed germination of S. khasianum and S. indicum reveal that gamma-irradiation enhances the rate of germination and percentage germination in the former and inhibits these aspects in the latter. Gamma-ray-induced stimulation and inhibition of seed germination are known in literature (Gunckel and Sparrow, 1961; Grover and Dhanju, 1979). Stimulatory effects of gamma-irradiation on germination may be due to shortening or breaking of dormancy period (Sparrow, 1966; Süss, 1966; Tavčar, 1966), structural changes in membranes (Elkeles, 1962), increased permeability of membranes to water and ions (Bacq and Herve, 1952; Hooker *et al.*, 1958; Skou, 1963). It may also be due to increase in the oxygen uptake of seeds thus causing formation of peroxy radicals in the tissues which in turn may inactivate the inhibitors by oxidation and thus tilting the promoter/inhibitor balance in favour of promoters (Maherchandani, 1976). Radiation-induced inhibition of seed germination is attributed to the inactivation/destruction of germination regulators in the seeds (Gordon and Weber, 1955; Haber and Luipold, 1959). Dnyansagar and Tarar (1971), however, are of the opinion that while lower exposures of radiation stimulate seed germination by inducing early DNA synthesis, the higher exposures inhibited both seed germination and DNA synthesis. Khanna and Maherchandani (1980) consider that the disturbances in the biosynthetic pathways in irradiated seeds at later stages possibly cause the production of insufficient amount of enzymes which in turn result in reduced growth and development of such plants.

Both in *S. indicum* and *S. khasianum* although lower exposures of gamma rays did not stimulate seedling survival, the higher exposures were inhibitory and the inhibition increased with increasing exposure. In *S. khasianum* only 25 and 30 kR treatments caused a significant decrease in seedling survival while in *S. indicum* 20 kR and above inhibited seedling survival drastically. Thus the minimum exposure required for inducing inhibition to seedling survival differed in the two species. Increased inhibition of seedling survival by higher exposures of radiation is reported for other plants also (Fujii and Matsumara, 1958; Prasad and Godward, 1975; Torne and Desai, 1975).

In *S. khasianum* even though 2.5 - 10 kR exposures of gamma-rays did not improve seedling survival the total seedling availability increased since the gamma-ray exposures (2.5 - 10 kR) induced significant increase in seed germination. In *S. indicum*, however, both these aspects were inhibited by gamma rays and therefore number of seedlings available was less compared to unirradiated control.

It is interesting that the two species *S. khasianum* and *S. indicum* have displayed a differential response to gamma-irradiation with reference to seed germination, wherein gamma rays have significantly stimulated seed germination in the former and inhibited in the latter. These results are in agreement with the findings in *Papaver* (Grover and Dhanju, 1979).

CHAPTER IV

GROWTH AND YIELD

INTRODUCTION

Ionizing radiations are being increasingly used for the improvement of crop plants. Kapoor and Datta (1967) have reported gamma-ray-induced useful mutations in certain medicinal and aromatic plants. Though the genetic improvement of *Solanum khasianum* with gamma rays has been attempted (Bhatt, 1972; Chauhan *et al*., 1975a; Chauhan *et al*., 1976), the success has not been great. In a radiation based crop improvement programme the mutagenic efficiency is determined to a considerable extent by the degree of M_1 injuries (Gaul, 1964; Ehrenberg, 1971) and such injuries have a bearing on the size of M_1 population to be treated and the M_2 to be raised (Blixt, 1972). Further, in a 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 the different species (Sparrow *et al*., 1961a, b) still information on radiosensitivity of different species under comparable conditions is of value (Rudolph, 1971) and help in understanding the radiobiological responses of the species.

Since information on the above aspects are lacking for these two species an attempt has been made to analyse the effect of different acute exposures of gamma rays on growth and yield in *S. khasianum* and *S. indicum*.

MATERIALS AND METHODS

The details of seed irradiation are given in Chapter III. Since exposures of 25 kR and above drastically inhibited seed germination and seedling survival, only seedlings obtained from seeds given 2.5, 5, 10, 15 and 20 kR exposures were used for studying the gamma-ray effects on growth, development and yield of the two species.

Seedlings exhibiting chimeric leaves and leaf abnormalities were scored at 60 days after seed sowing; before transplanting seedlings to polythene sleeves. One and a half month old seedlings were transplanted in polythene sleeves (5" x 10") containing 1:1 mixture of soil and farmyard manure. After a month these seedlings were transplanted in the experimental plots of North-Eastern Hill University, Shillong. The distance between plants was 50 cms and between rows 70 cms. The treatments were randomized and for each treatment ten plants were maintained.

Observations on growth, reproductive characters and yield aspects of control and irradiated plants of the two species were recorded from 10 plants per treatment. Plant height, plastochron index, maximum length and breadth of sixth leaf, spine number and size on the dorsal and ventral surfaces of sixth leaf were recorded in 90 day old seedlings just before the visible appearance of flower buds. Plant height was measured as the distance from cotyledonary node to shoot tip. Plastochron index was calculated according to the formula

$$\mathrm{Pi} = n + \frac{\log Ln - \log 10}{\log Ln - \log Ln + 1}$$

where n = number of leaves with a length exceeding 10 mm; logLn is length of that leaf which just exceeds 10 mm; logLn + 1 is the length of that leaf which is less than 10 mm.

Leaf area was calculated by multiplying length with breadth. Spine frequency (intensity) per unit area was calculated by dividing spine number with leaf area. Spine size was determined by measuring all the spines on dorsal and ventral surfaces of the sixth leaf.

At the time of harvest plant height was again recorded by measuring the distance from cotyledonary node to tip of the longest branch. Number of branches (primary and secondary) per plant were counted. The berries were harvested plant wise and their number and weight determined.

Berry diameter, volume and number of seeds per berry were determined from 20 batches of 5 berries each per treatment.

For estimation of glycoalkaloid content the method followed by Crusena *et al.* (1965) was used in this study. Three replicates per treatment were run. The berries of *S. khasianum* (yellow) and *S. indicum* (red) were harvested and dried in a hot air oven around 60°C. Two grammes of the dried pulverized berry were covered with 30 ml of a mixture of ethanol, glacial acetic acid and water (50:2:48) and left for six hours with periodic shaking. The extract was filtered and the residue on filter paper was washed three times with 5 ml of the above mentioned mixture. The filtrates were combined and transferred to a beaker and evaporated on a water bath to half its volume. It was then filtered and the residue on filter paper was washed 4 times with 6 ml of 2% acetic acid. The filtrates were combined and the glycoalkaloids were precipitated from acetic acid solution by adding 25% solution of NH_4OH to an alkaline reaction using phenolphthalein as an indicator. The mixture was heated to 60-70°C and left overnight to coagulate the precipitate. The precipitate thus formed was filtered off and the residue on the filter paper was washed with 100 ml of 0.1% NH_4OH solution, until the washings were colourless. The washed precipitate was dissolved on the filter paper in 10 ml of a mixture of ethanol, glacial acetic acid and water (4:1:5). The filtrate was transferred to a volumetric flask and the volume was made upto 100 ml with the 4:1:5 mixture. One ml of this extract was diluted with 1 ml of 4:1:5. This solution in a test tube was kept in a container containing ice and 4 ml of concentrated sulphuric acid was added over a period of 2 minutes to this ice cold solution. Then 2 ml of 1% formalin was added over a period of 1 minute. Thereafter a crimson coloured solution was formed whose optical density was measured at 490 nm in a systronic colorimeter. The optical density readings were matched with the standard graph and the solasodine content in 2 ml of the sample was obtained. The final results tabulated were calculated on the basis of solasodine amount per 100 gms of dry berry weight.

The standard graph prepared was linear and obtained by using different concentrations of pure solasodine (M/s. Organon Limited). The procedure followed was same as outlined above. All the chemicals used in this study were of analytical grade.

RESULTS

S. khasianum :

The results of the effect of gamma rays on plant height, plastochron index, number of branches and sixth leaf are given in tables 3-5. Gamma-ray exposures had no effect on plant height both at 90 days after sowing and at maturity. Plastochron index was inhibited by higher exposures (15 and 20 kR) (Table 3). Though all gamma-ray exposures stimulated branches per plant the differences were significant from control in 5-15 kR irradiated plants (Fig. 2).

Normal leaves of *S. khasianum* are ovate with slight lobing of margins (Plate 1 a). The various abnormalities observed were: leaves with unequal lamina and clefting of leaf lamina both towards the top and sides (Plate 1 b-d). The incidence of abnormal shaped leaves was exposure dependent (Table 4).

The node number having leaf abnormalities increased with gamma-ray exposures and 15 and 20 kR exposures resulted in leaf abnormalities persisting upto 5th node (Table 4).

Table 3: Gamma-ray effects on the growth of *Solanum khasianum* plants 90 days after sowing and at maturity.

Treatment	90 DAYS AFTER SOWING		AT MATURITY	
	Plant height (cm)	Plastochron index	Plant height (cm)	Number of branches
CONTROL	9.2 ± 0.31	11.7 ± 0.23	124.6 ± 4.2	30.0 ± 9.2
2.5 kR	9.3 ± 0.63 (+1.00)	12.1 ± 0.28 (+3.41)	124.0 ± 5.4 (-0.48)	35.6 ± 3.5 (+18.66)
5.0 kR	9.4 ± 0.48 (+2.17)	11.8 ± 0.32 (+0.85)	124.2 ± 6.3 (-0.32)	42.3* ± 4.9 (+41.00)
10.0 kR	9.1 ± 0.62 (-1.08)	11.1 ± 0.32 (-5.12)	128.2 ± 5.8 (+2.88)	53.7* ± 2.9 (+79.00)
15.0 kR	8.7 ± 0.23 (-5.43)	10.7* ± 0.20 (-8.54)	132.4 ± 5.2 (+6.26)	50.2* ± 1.4 (+67.33)
20.0 kR	8.0 ± 0.37 (-13.04)	10.7* ± 0.27 (-8.54)	126.6 ± 5.0 (+1.60)	36.9 ± 2.7 (+23.00)
L.S.D.(p=0.05)		0.84		10.00

± S.E.

* Significantly different from control (p=0.05)

Figures in bracket represent percent stimulation (+)/inhibition (-) over control

Fig. 2: Gamma-ray effects on number of branches in *S. khasianum* and *S. indicum* plants.

Fig. 2

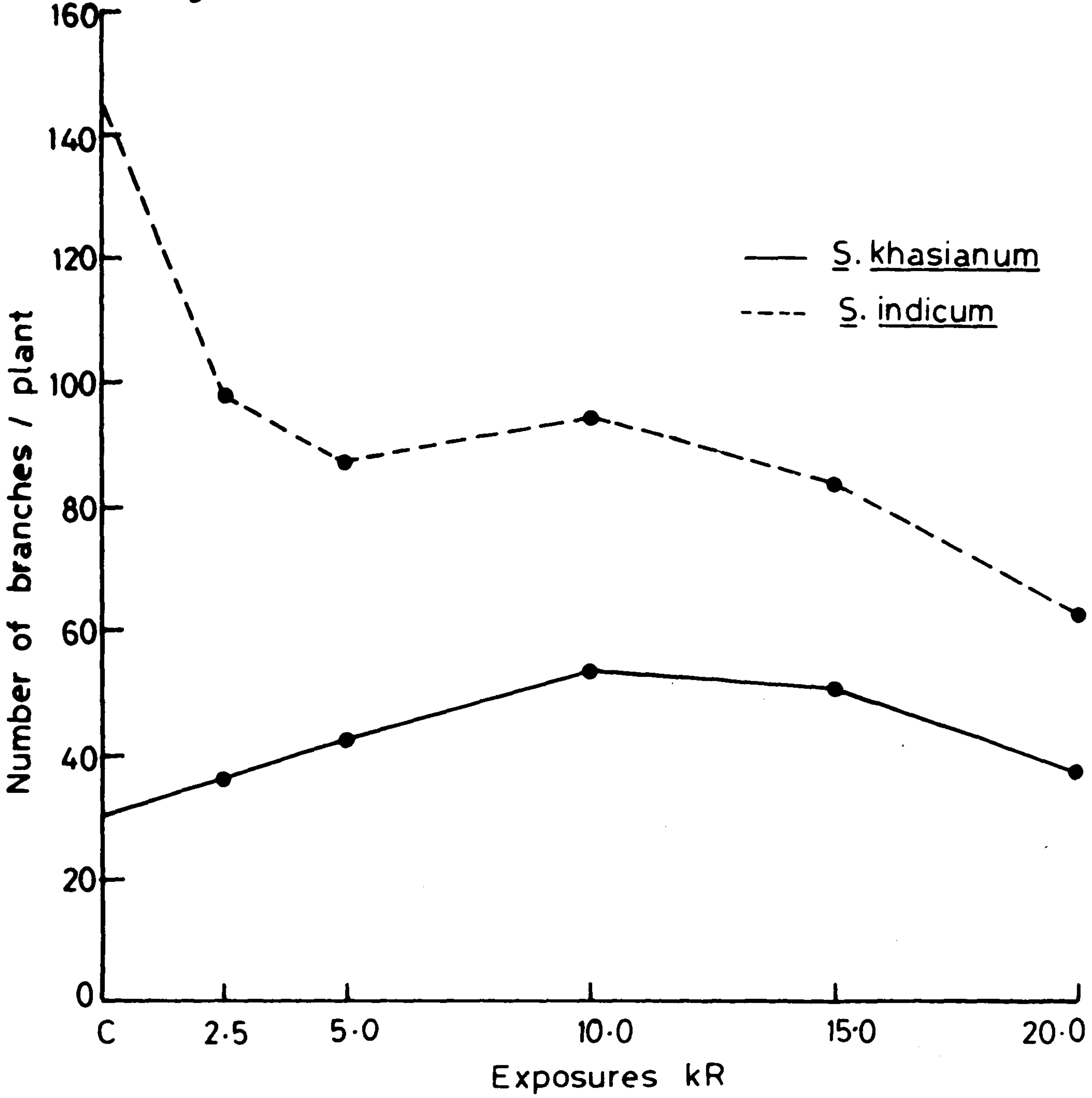

Number of branches / plant
160
140
120
100
80
60
40
20
0
C
2·5
5·0
10·0
15·0
20·0
Exposures kR
S. khasianum
S. indicum

Plate 1 : *Variation in leaf shape and leaves with* chlorophyll chimeras in control and irradiated S. khasianum.

a - d: Variation in leaf shape.

e - f: Chimeric leaves.

PLATE 1

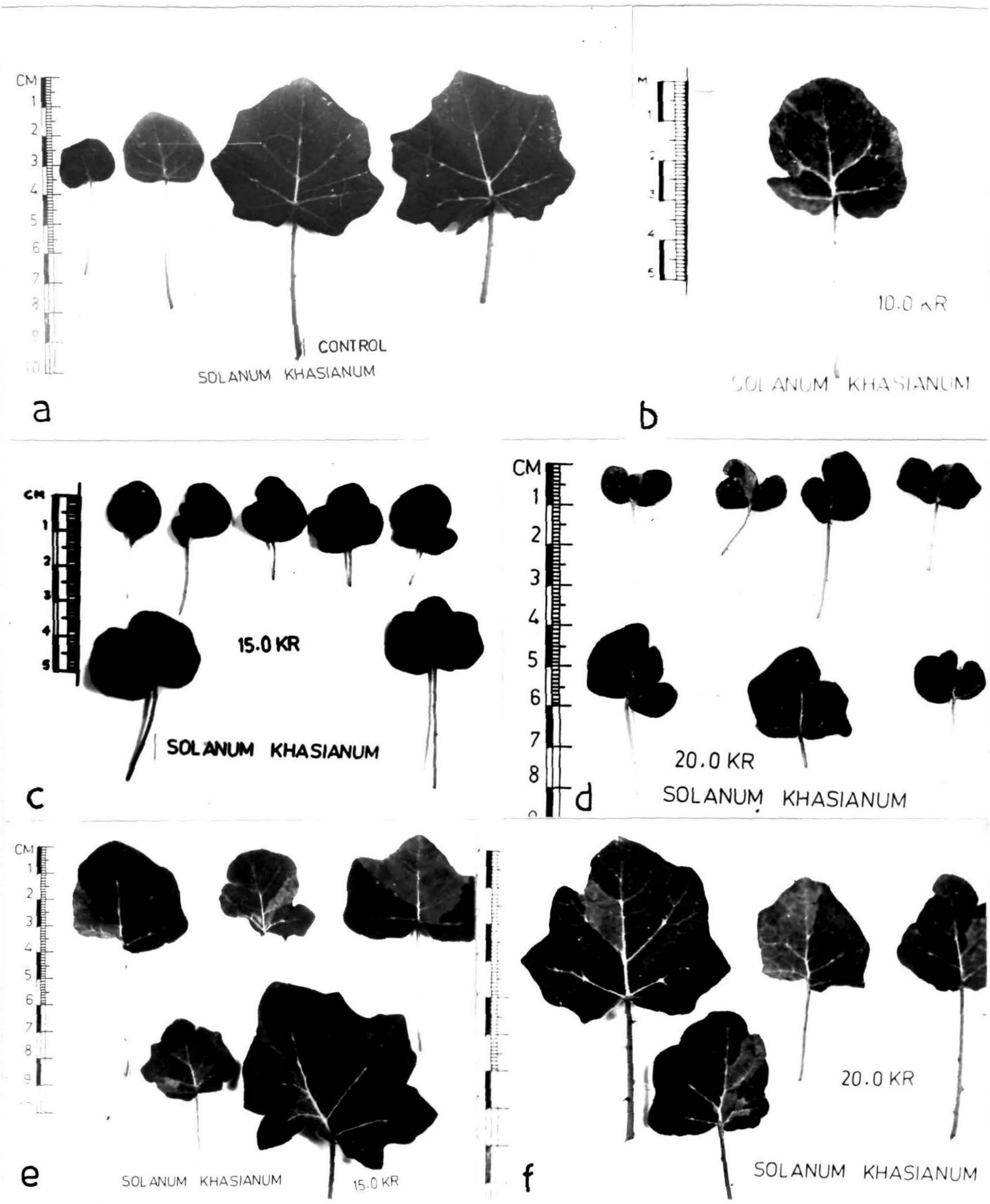

CM
CONTROL
SOLANUM KHASIANUM
a
10.0 KR
SOLANUM KHASIANUM
b
CM
15.0 KR
SOLANUM KHASIANUM
c
CM
20.0 KR
SOLANUM KHASIANUM
d
CM
SOLANUM KHASIANUM
15.0 KR
e
20.0 KR
SOLANUM KHASIANUM
f

Table 4: Gamma-ray effects on leaf abnormalities and chlorophyll chimeras in <u>Solanum khasianum</u> seedlings, 60 days after sowing.

Treatment	Total number of plants	Number of plants with leaf abnormalities	Number of plants with leaf chimeras	Node number upto which leaf abnormalities persisted	% of plants with leaf abnormalities	% of plants with chimeric leaves
CONTROL	150	0	1	1	0.0	0.66
2.5 kR	196	10	3	3	5.1	1.53
5.0 kR	200	11	4	3	5.5	2.00
10.0 kR	225	11	42	4	4.8	18.60
15.0 kR	182	14	44	5	7.6	24.10
20.0 kR	150	11	39	5	7.3	26.00

Table 5: Gamma-ray effects on sixth leaf of <u>Solanum khasianum</u> plants, 90 days after sowing.

	SIXTH LEAF				DORSAL SURFACE		VENTRAL SURFACE	
Treatment	Length (cm)	Breadth (cm)	Area (cm^2)	Spine intensity	Spine number	Spine size(mm)	Spine number	Spine size(mm)
CONTROL	5.7±0.3	6.8±0.4	38.76	0.84	15.2±0.8	4.1±0.2	17.4±1.0	3.1±0.2
2.5 kR	5.0±0.3 (-12.28)	6.0±0.4 (-11.76)	29.4 (-24.14)	1.0 (+19.0)	13.7±0.9 (-9.86)	3.7±0.3 (-9.75)	15.7±1.2 (-9.77)	3.2±0.2 (+3.22)
5.0 kR	5.4±0.3 (-5.26)	7.3±0.1 (+7.35)	39.42 (+1.70)	0.89 (+5.95)	16.6±0.9 (+9.21)	3.7±0.3 (-9.75)	18.8±1.0 (+8.04)	3.1±0.2 (0.0)
10.0 kR	5.0±0.4 (-12.28)	6.4±0.5 (-5.88)	32.0 (-17.44)	0.91 (+8.33)	14.0±0.6 (-7.89)	3.6±0.2 (-12.1)	15.3±1.1 (-12.06)	3.0±0.2 (-3.22)
15.0 kR	4.2*±0.3 (-26.31)	5.2*±0.2 (-23.52)	21.84 (-43.65)	1.16 (+38.0)	11.4*±0.7 (-25.0)	3.3±0.2 (-19.51)	14.0±0.8 (-19.54)	2.4*±0.2 (-22.58)
20.0 kR	4.5*±0.3 (-21.05)	5.7*±0.4 (-16.17)	25.65 (-33.82)	0.78 (-7.14)	10.1*±0.7 (-33.55)	3.5±0.3 (-14.63)	10.0*±1.0 (-42.52)	2.6*±0.2 (-16.12)
L.S.D. (p=0.05)	0.79	0.95			2.85		3.86	0.46

± S.E.

* Significantly different from control (p=0.05)

Figures in bracket represent percent stimulation (+)/inhibition (-) over control

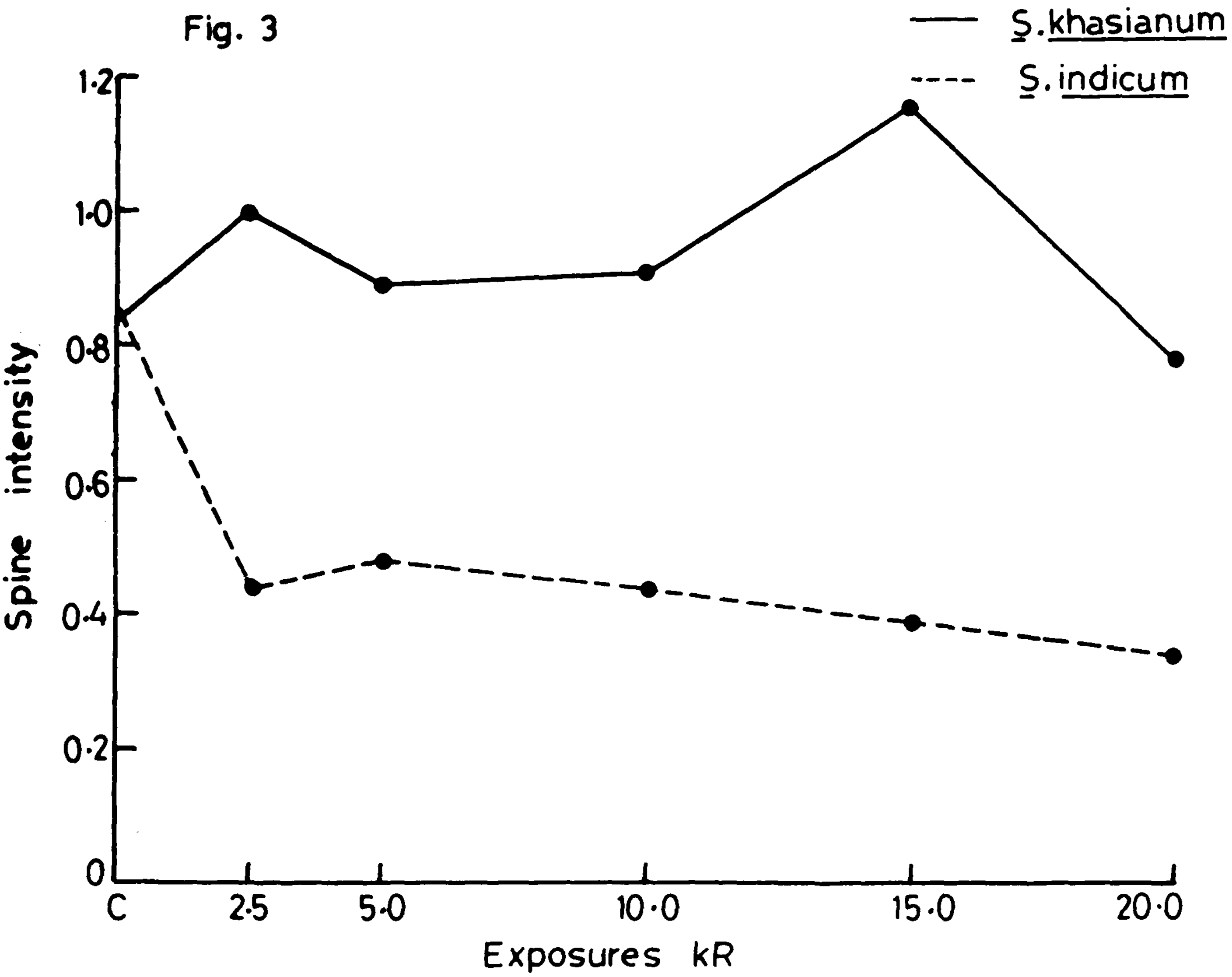
The production of leaves exhibiting chlorophyll chimeras (Plate 1,e,f) showed an increase with increasing gamma-ray exposures (Table 4).

All exposures of gamma rays inhibited leaf length but the inhibitions were significant in 15 and 20 kR exposures alone. Leaf breadth which was not influenced by low exposures of gamma rays, was inhibited significantly in plants given 15 and 20 kR exposures (Table 5). Leaf area was inhibited by all the gamma-ray treatments except 5 kR.(Table 5). Compared to control plants, leaves of gamma irradiated plants were more spiny except in the plants given 20 kR exposure (Table 5; Fig. 3).

Analysis of spines on dorsal and ventral surfaces of the sixth leaf revealed that compared to the dorsal surface more spines are produced on the ventral surface (Table 5). While low exposures of gamma rays did not affect spine number of both dorsal and ventral surface in the sixth leaf of irradiated plants, higher exposures (15 and 20 kR) inhibited spine production. The gamma-ray-induced injury to spine number was more on ventral surface than on dorsal surface (Table 5). Compared to control, though irradiated plants had smaller spines, the differences were significant only in the case of ventral surface spines produced on leaves of plants exposed to 15 and 20 kR (Table 5; Fig. 4). Thus the ventral surface spines were more sensitive to gamma rays than spines present on the dorsal surface of leaves.

Fig. 3: Gamma-ray effects on spine intensity of the sixth leaf in *S. khasianum* and *S. indicum*.

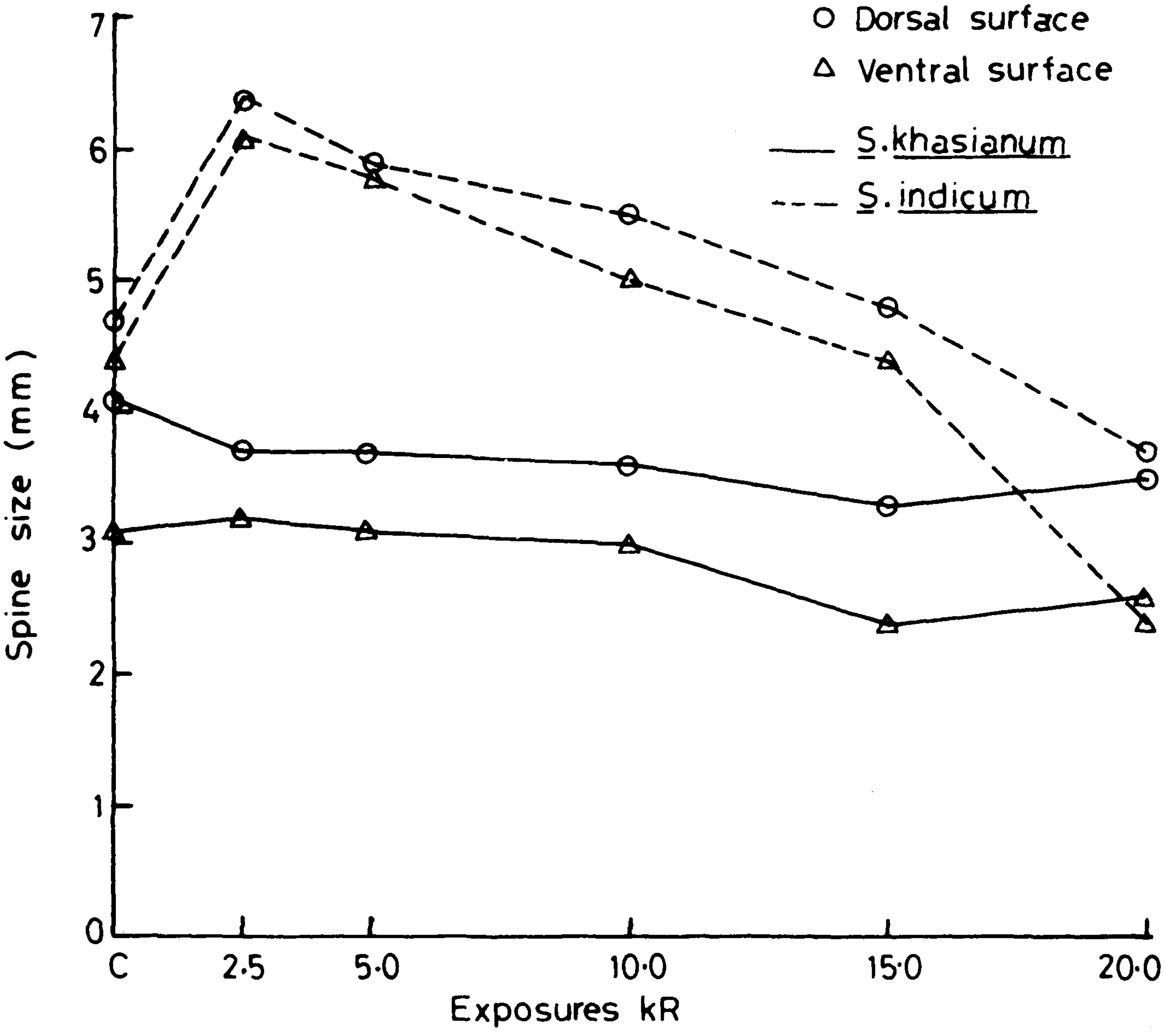
Fig. 3



S. khasianum
S. indicum
Spine intensity
1·2
1·0
0·8
0·6
0·4
0·2
0
C
2·5
5·0
10·0
15·0
20·0
Exposures kR

Fig. 4: Gamma-ray effects on the size of dorsal and ventral surface spines in the sixth leaf of *S. khasianum* and *S. indicum* plants.

Fig. 4



Dorsal surface
Ventral surface
S. khasianum
S. indicum
Spine size (mm)
0
1
2
3
4
5
6
7
C
2·5
5·0
10·0
15·0
20·0
Exposures kR

Table 6: Gamma-ray effects on yield and alkaloid content in <u>Solanum khasianum</u> plants.

Treatment	Berry number	Per Berry weight (gms)	Berry yield (gms)	Alkaloid content (percent)
CONTROL	113 ± 12.2	6.84 ± 0.15	770.86 ± 83.76	0.53 ± 0.03
2.5 kR	136 ± 20.4 (+20.23)	7.28 ± 0.14 (+6.43)	986.44 ±148.93 (+27.96)	0.44 ± 0.04 (-16.98)
5.0 kR	152 ± 20.3 (+34.60)	6.82 ± 0.25 (-0.29)	1034.59 ±138.45 (+34.21)	0.52 ± 0.06 (-1.88)
10.0 kR	109 ± 6.7 (-3.10)	6.95 ± 0.19 (+1.60)	758.94 ± 46.96 (-1.54)	0.62 ± 0.03 (+16.98)
15.0 kR	104 ± 8.3 (-7.89)	7.01 ± 0.33 (+2.48)	727.63 ± 58.8 (-5.6)	0.51 ± 0.04 (-3.77)
20.0 kR	89 ± 4.7 (-20.85)	5.90* ± 0.31 (-13.74)	526.28 ± 28.22 (-31.72)	0.66* ± 0.05 (+24.52)
L.S.D. (p=0.05)		0.81		0.12

± S.E.

* Significantly different from control (p=0.05)

Figures in bracket represent percent stimulation (+)/inhibition (-) over control

Table 7: Gamma-ray effects on <u>Solanum khasianum</u> berries.

Treatment	Berry diameter (cm)	Berry volume (cm3)	Seed number per berry
CONTROL	2.69 ± 0.02	11.1 ± 0.33	377 ± 21
2.5 kR	2.81 ± 0.03	12.4 ± 0.33	381 ± 14
5.0 kR	2.78 ± 0.02	12.0 ± 0.32	393 ± 10
10.0 kR	2.67 ± 0.06	10.7 ± 0.70	358 ± 13
15.0 kR	2.71 ± 0.04	11.1 ± 0.54	319 ± 21
20.0 kR	1.99* ± 0.06	7.5* ± 0.38	309 ± 28
L.S.D. (p=0.05)	0.27	1.33	

± S.E.

* Significantly different from control (p=0.05)

The effect of gamma rays on berry number, yield and alkaloid content are given in table 6. Control plants produced 113 berries per plant. In gamma irradiated plants the number of berries increased upto 5 kR subsequent to which the number declined. However, the berry number in treated plants did not differ significantly from control (Table 6). The weight per berry while in plants given 2.5-15 kR exposures did not differ from control, the berries produced on plants given 20 kR exposure had significantly less weight (Table 6). Though in none of the treatments berry yield differed significantly from control, the plants given 2.5 and 5 kR exposure yielded more berries and plants raised from 10-20 kR exposed seeds yielded less berries (Table 6). The berries of control, which had a diameter of 2.69 cm., displaced a volume of 11.1 cc. and contained 377 seeds per berry (Table 7). The gamma-ray exposures of 2.5-15 kR had no appreciable effect on berry diameter and volume. However, berries from 20 kR plants were smaller and displaced lesser volume (Table 7). The various gamma-ray exposures had no marked effect on the seed number per berry (Table 7). The berries of control plants contained 0.53% of alkaloid on percent dry weight basis. The various gamma-ray exposures had no appreciable influence on the glycoalkaloid content except 20 kR which resulted in a significant increase in glycoalkaloid content as compared to control (Table 6; Fig. 5).

S. indicum :

The results of the effect of gamma-ray exposures on growth and sixth leaf characters of S. indicum are given in tables 8-10. A perusal of the table 8 reveals that gamma-ray exposures of 5 kR and above significantly inhibited plant height at 90 days after sowing, but at maturity, the plant height did not differ appreciably from control. Plastochron index was inhibited by higher exposures (15 and 20 kR) (Table 8). All gamma-ray treatments inhibited number of branches per plant (Table 8; Fig. 2).

Normal leaves were ovate with slight lobing of margins (Plate 2 a). In the various gamma-ray treatments the leaf abnormalities observed were the production of leaves with unequal lamina and clefting of lamina both towards tip and sides (Plate 2 b-f). The production of such leaves increased with increasing gamma-ray exposures (Table 9).

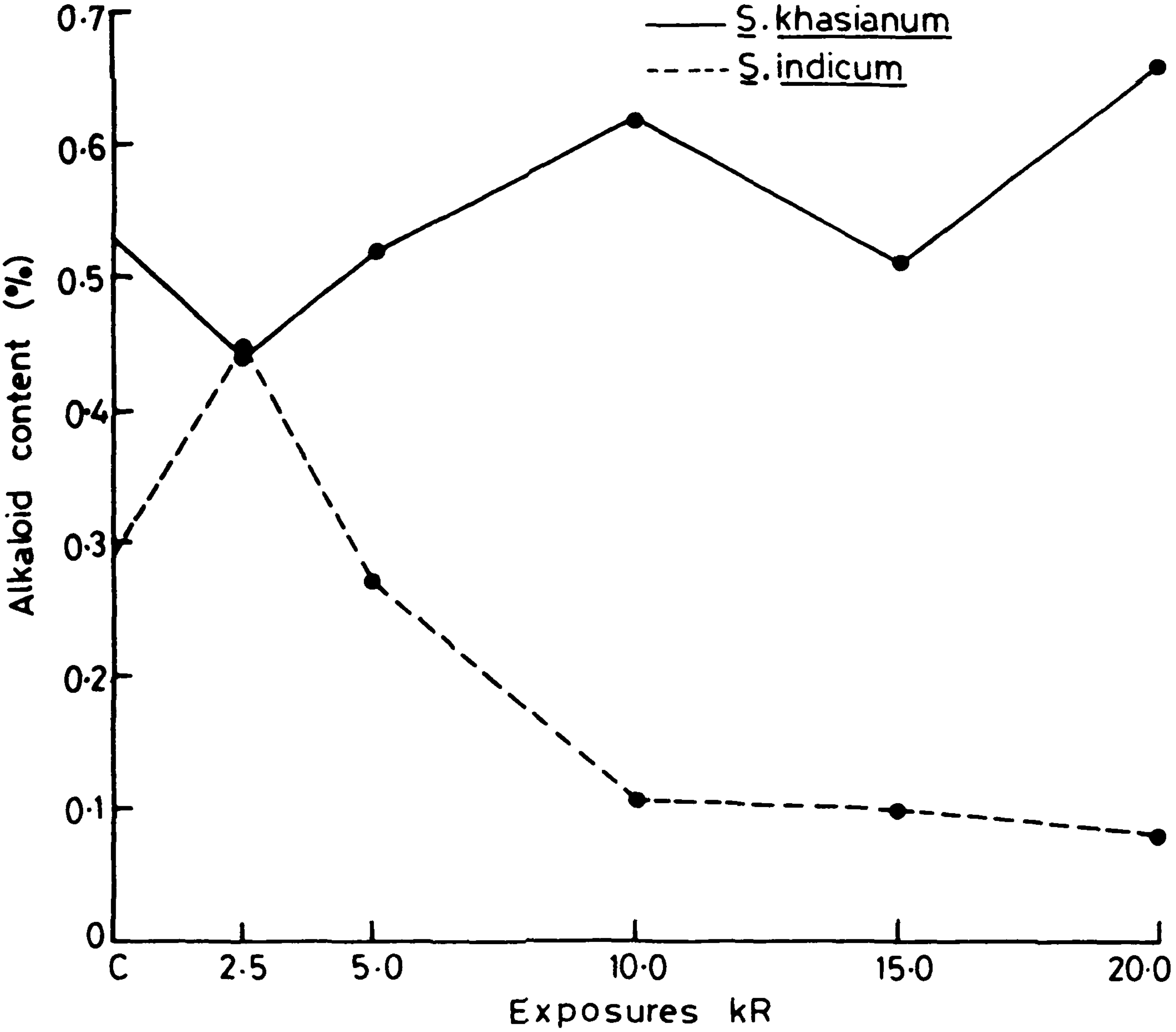
The node number having leaf abnormalities increased with gamma-ray exposures and in 20 kR exposure leaf abnormalities persisted upto the 6th node (Table 9).

The production of leaves with chlorophyll chimeras (Plate 2 b,c,e,f) was exposure dependent (Table 9) and increased with increasing exposure.

The effect of gamma-ray exposures on leaf expansion revealed that only 20 kR exposure could cause significant reduction of leaf length (Table 10). The lateral expansion of sixth leaf was significantly stimulated in 2.5 kR exposure, the other exposures, however, had no effect on lateral expansion of

Fig. 5: Gamma-ray effects on alkaloid content of berries of S. *khasianum* and S. *indicum* plants.

Fig. 5



S. khasianum
S. indicum
Alkaloid content (%)
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
C
2·5
5·0
10·0
15·0
20·0
Exposures kR

Plate 2: : Variation in leaf shape and leaves with chlorophyll chimeras in control and irradiated *S. indicum*.

a - f: Variation in leaf shape.
b,c,e,f: Chimeric leaves.

PLATE 2

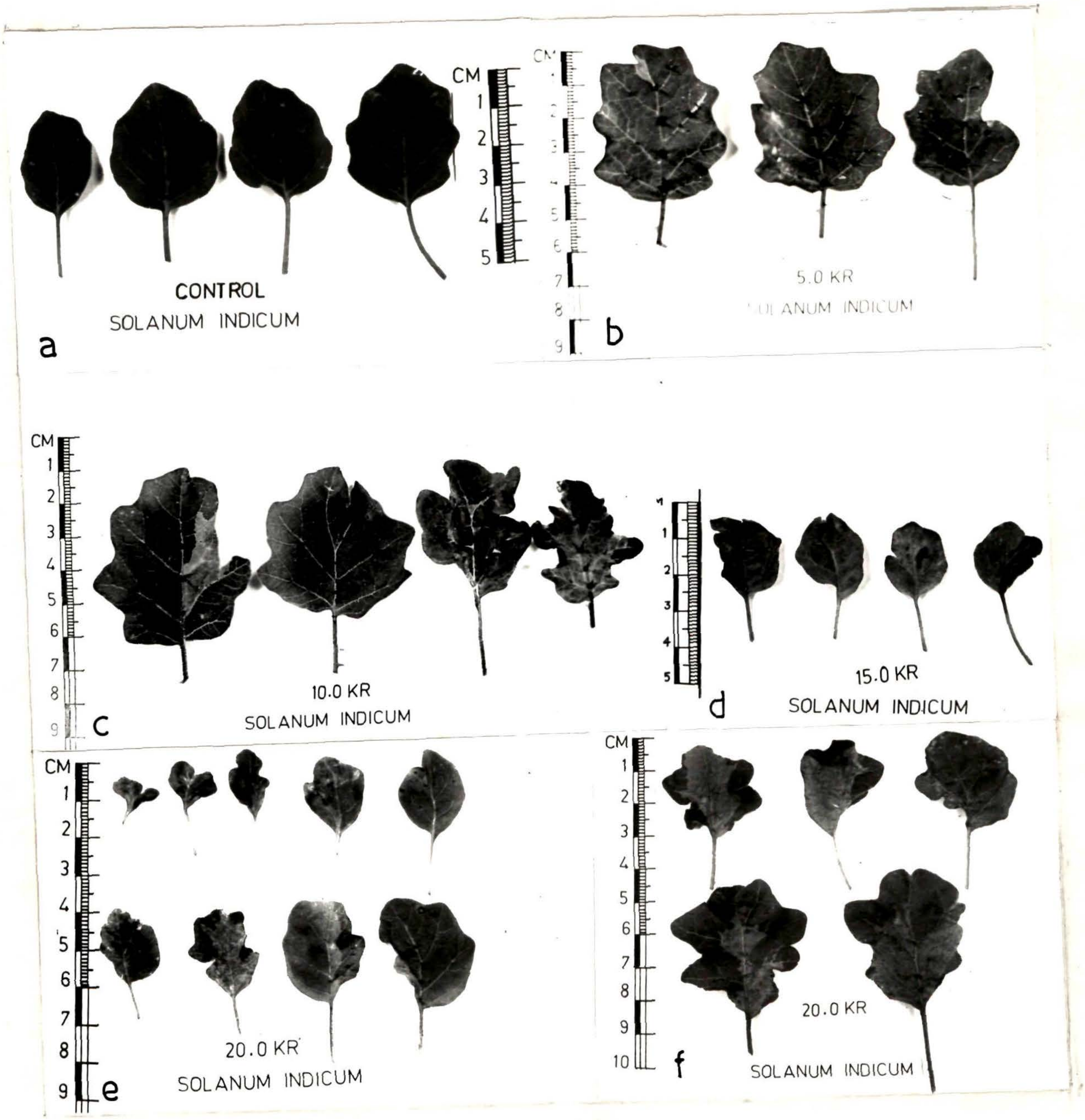

CM
CONTROL
SOLANUM INDICUM
a
CM
5.0 KR
SOLANUM INDICUM
b
CM
10.0 KR
SOLANUM INDICUM
c
15.0 KR
SOLANUM INDICUM
d
CM
20.0 KR
SOLANUM INDICUM
e
CM
20.0 KR
SOLANUM INDICUM
f

Table 8: Gamma-ray effects on the growth of Solanum indicum plants 90 days after sowing and at maturity.

	90 DAYS AFTER SOWING		AT MATURITY	
Treatment	Plant height (cm)	Plastochron index	Plant height (cm)	Number of branches
CONTROL	4.9 ± 0.35	8.7 ± 0.40	106.3 ± 7.8	145.3±28.7
2.5 kR	5.2 ± 0.27 (+6.12)	8.6 ± 0.42 (-1.14)	99.5 ± 7.1 (-6.39)	98.0±14.3 (-32.55)
5.0 kR	3.7* ± 0.14 (-24.48)	8.3 ± 0.26 (-4.59)	91.5 ± 4.5 (-13.92)	86.9± 7.4 (-40.19)
10.0 kR	4.1* ± 0.18 (-16.32)	7.8 ± 0.29 (-10.34)	99.8 ± 6.1 (-6.11)	94.4± 9.6 (-35.03)
15.0 kR	3.2* ± 0.22 (-34.69)	7.5* ± 0.20 (-13.79)	98.3 ± 5.4 (-7.52)	83.6*±15.8 (-42.46)
20.0 kR	2.6* ± 0.22 (-46.93)	6.2* ± 0.43 (-28.73)	80.3 ± 5.8 (-24.45)	62.5*±13.4 (-56.98)
L.S.D.(p=0.05)	0.74	1.07		51.9

± S.E.

* Significantly different from control (p=0.05)

Figures in bracket represent percent stimulation (+)/inhibition (-) over control

Table 9: Gamma-ray effects on leaf abnormalities and chlorophyll chimeras in *Solanum indicum* seedlings, 60 days after sowing.

Treatment	Total number of plants	Number of plants with leaf abnormalities	Number of plants with leaf chimeras	Node number upto which leaf abnormalities persisted	% of plants with leaf abnormalities	% of plants with chimeric leaves
CONTROL	242	0	0	-	0.0	0.0
2.5 kR	227	2	16	4	0.8	7.0
5.0 kR	223	9	29	5	4.0	13.0
10.0 kR	218	28	61	5	12.8	27.9
15.0 kR	203	25	63	5	12.3	31.0
20.0 kR	194	27	63	6	13.9	32.4

Table 10: Gamma-ray effects on sixth leaf of <u>Solanum indicum</u> plants, 90 days after sowing.

Treatment	SIXTH LEAF Length (cm)	Breadth (cm)	Area (cm²)	Spine intensity	DORSAL SURFACE Spine number	Spine size(mm)	VENTRAL SURFACE Spine number	Spine size(mm)
CONTROL	4.9±0.6	3.9±0.5	19.11	0.85	10.5±1.0	4.7±0.2	5.9±0.8	4.4±0.3
2.5 kR	5.7±0.2 (+16.32)	5.1±0.3* (+30.76)	29.0 (+51.83)	0.44 (-48.23)	8.6±0.9 (-18.09)	6.4±0.3* (+36.17)	4.2±0.5 (-28.81)	6.1±0.3* (+38.63)
5.0 kR	4.9±0.1 (0.0)	4.3±0.2 (+10.25)	21.0 (+9.94)	0.48 (-43.52)	6.9±0.6 (-34.28)	5.9±0.4 (+25.53)	3.2±0.4* (-45.76)	5.8±0.4 (+31.81)
10.0 kR	5.2±0.2 (+6.12)	4.5±0.2 (+15.38)	23.4 (+21.51)	0.44 (-48.23)	7.2±0.7 (-31.42)	5.5±0.4 (+17.02)	3.1±0.5* (-47.45)	5.0±0.4 (+13.63)
15.0 kR	4.0±0.3 (-18.36)	3.6±0.3 (-7.69)	14.4 (-24.60)	0.39 (-54.11)	3.8±0.7* (-63.80)	4.8±0.6 (+2.12)	1.9±0.3* (-67.69)	4.4±0.6 (0.0)
20.0 kR	3.7±0.1* (-24.48)	3.3±0.2 (-15.38)	12.2 (-36.12)	0.34 (-60.0)	3.3±0.7* (-68.57)	3.7±0.5 (-21.27)	0.9±0.2* (-84.74)	2.4±0.5* (-45.45)
L.S.D. (p=0.05)	1.17	1.14			4.28	1.45	1.77	1.59

± S.E.

* Significantly different from control (p=0.05)

Figures in bracket represent percent stimulation (+)/inhibition (-) over control

leaf (Table 10). Leaf area was stimulated by low exposures (2.5, 5 and 10 kR) but the higher exposures (15 and 20 kR) were inhibitory (Table 10). All gamma-ray treatments reduced spine intensity (Table 10; Fig. 3).

Analysis of spines on dorsal and ventral surfaces of sixth leaf in control plants revealed that more spines are produced on the dorsal surface. However, the gamma-ray-induced injury to spine number was more on ventral surface than dorsal surface (Table 10). Spine size of both the dorsal and ventral surfaces, which was stimulated by 2.5 kR exposure of gamma rays, did not differ from control in plants given other exposures of gamma rays (5-15 kR) (Table 10). In 20 kR exposed plants, though the ventral surface spines were smaller than control, the spines on the dorsal surface remained unaffected (Table 10; Fig. 4).

The effect of gamma rays on berry number, yield and alkaloid content are given in table 11. The unirradiated control plants produced maximum number of berries (Table 11) and in irradiated plants the berry number decreased with an increase in the gamma-ray exposure. Though the diffefences were significant even in 10 kR irradiated plants, more than 50% reduction in berry number was induced by 15 and 20 kR exposures (Table 11). Berry weight was significantly reduced vis-a-vis control in 10, 15 and 20 kR irradiated plants. But only 20 kR exposure could cause more than 50% reduction in berry weight (Table 11). The decrease in berry yield vis-a-vis control was significant from 5 kR onwards and more than 50% reduction in berry yield occurred in

Table 11: Gamma-ray effects on yield and alkaloid content in <u>Solanum indicum</u>.

Treatment	Berry number	Per Berry weight (gms)	Berry yield (gms)	Alkaloid content (percent)
CONTROL	420 ± 94.9	0.4084 ± 0.0225	171.68 ± 38.77	0.29±0.02
2.5 kR	306 ± 42.1 (-27.16)	0.3910 ± 0.0198 (-4.26)	119.72 ± 16.47 (-30.26)	0.45*±0.03 (+55.17)
5.0 kR	273 ± 60.0 (-34.99)	0.3546 ± 0.0185 (-13.17)	96.90* ± 21.27 (-43.55)	0.27±0.002 (-6.89)
10.0 kR	214* ± 36.3 (-49.09)	0.2633* ± 0.0359 (-35.52)	56.34* ± 9.56 (-67.18)	0.11*±0.01 (-62.06)
15.0 kR	175* ± 47.5 (-58.27)	0.2700* ± 0.0316 (-33.88)	47.35* ± 12.82 (-72.41)	0.10*±0.01 (-65.51)
20.0 kR	171* ± 52.4 (-59.22)	0.1458* ± 0.0363 (-64.29)	24.98* ± 7.64 (-85.44)	0.08*±0.009 (-72.41)
L.S.D. (p=0.05)	176.7	0.0865	62.59	0.065

± S.E.

* Significantly different from control (p=0.05)

Figures in bracket represent percent stimulation (+)/inhibition (-) over control

Table 12: Gamma-ray effects on *Solanum indicum* berries.

Treatment	Berry diameter (cm)	Berry volume (cm^3)	Seed number per berry
CONTROL	0.98 ± 0.01	0.56 ± 0.01	41 ± 1.0
2.5 kR	0.96 ± 0.01	0.51 ± 0.02	36* ± 1.7
5.0 kR	0.92* ± 0.01	0.47* ± 0.01	33* ± 1.6
10.0 kR	0.86* ± 0.01	0.41* ± 0.01	24* ± 1.6
15.0 kR	0.87* ± 0.02	0.45* ± 0.02	30* ± 2.3
20.0 kR	0.77* ± 0.02	0.33* ± 0.02	22* ± 1.9
L.S.D. ($p=0.05$)	0.05	0.059	4.8

± S.E.

* Significantly different from control ($p=0.05$)

10 kR plants. Thus berry yield is most radiosensitive in this species. The berries of control plants had a diameter of 0.98 cm, displaced a volume of 0.56 cc, and contained 41 seeds per berry (Table 12). Both berry size and berry volume were less in plants given an exposure of 5 kR or more (Table 12). All gamma-ray exposures reduced seed number per berry (Table 12). The berries of control plants yielded 0.29% of glycoalkaloid on dry weight basis. Except 2.5 kR exposure of gamma rays, which increased glycoalkaloid content, all other exposures (5-20 kR) inhibited the alkaloid content of the berries (Table 11; Fig. 5).

DISCUSSION

A comparison of gamma-ray effects on plant height of S. khasianum and S. indicum revealed that while gamma irradiation did not inhibit plant height in the former, significant inhibition was caused in the latter. In both the species plastochron index was significantly inhibited, however. Gamma-ray-induced growth inhibition of solanaceous species has been reported in Lycopersicon (Sparrow and Singleton, 1953), Nicotiana (Meiselman, 1956). Radiation-induced reduction in seedling height and growth inhibition may be due to destruction or damage to apical meristems (Iqbal, 1969; Patel and Shah, 1974); partial failure of the internodes to elongate, decrease in the number of proliferating cells (Van't Hof and Sparrow, 1965); chromosome structural damage in meristematic cells following irradiation (Gray and Scholes, 1951); inactivation of growth substance (Skoog, 1934) and reduced auxin levels (Skoog, 1935). But according to Sparrow *et al.* (1956) the ascribing of reduced stem elongation to reduced auxin levels alone may be an oversimplification since nutritional levels and mechanisms of assimilation may also be important factors.

At maturity, in irradiated plants height did not differ from control in both the species. This could be due to the recovery from radiation damage. Recovery from radiation damage with the progression of time is reported in literature (Gunckel and Sparrow, 1961).

A comparison of gamma-ray effects on number of branches produced per plant in *S. khasianum* and *S. indicum* revealed that while gamma irradiation stimulated the production of branches in the former, significant inhibition was caused in the latter. Ionizing radiations are known to break apical dominance and accelerate lateral branches (Sax, 1963). According to Skoog (1935), irradiation of the shoot tips by relatively small doses of x-rays reduces or inactivates the auxin production and promotes development of lateral buds by releasing their arrest. Radiation-induced stimulation of side shoots is reported in literature (Kahan, 1973; Shamsi and Bajwa, 1978). In *S. indicum*, however, there was an exposure dependent reduction in the production of branches and more than 50% reduction in branch number vis-a-vis control was observed in 20 kR plants. Radiation-induced inhibition of lateral branching has been reported in chillies (Sethupathi, 1976) and *S. khasianum* (Chauhan, 1978a).

The percentage of plants with chimeric leaves and leaf abnormalities were more in *S. indicum* than in *S. khasianum*, Thus *S. indicum* is more radiosensitive. In both the species the production of seedlings with abnormal leaves was found to increase with increasing gamma-ray exposures. Similar results have been reported for other plants also (Gunckel, 1957; Chauhan, 1969; Rai and Singh, 1976).

In both the species leaf length was more sensitive to gamma-ray exposures than leaf breadth. This could be due to greater injury to leaf tip meristem compared to the lateral meristem. Similar were the observations of Chauhan (1978a) in *S. khasianum*. Sixth leaf of the irradiated *S. khasianum* plants was smaller compared to control except in 5 kR plants which had marginally bigger leaves. Unlike this in *S. indicum* gamma-ray exposures upto 10 kR, stimulated leaf size, the higher exposures induced inhibition. Thus we find that compared to *S. khasianum* leaf expansion in *S. indicum* is more amenable to radiation treatments.

In the present study the sixth leaf of the irradiated plants of both the species had less spines compared to control. The only exception being *S. khasianum* plants given 5 kR exposure which bore leaves with marginally higher number of spines. However, the increase in spine number was not significant vis-a-vis control. In *S. khasianum* while only 15 and 20 kR exposures induced significant inhibition of spine number, in *S. indicum* even 5 kR exposure was inhibitory. Thus spine development in S. indicum is comparatively more radiosensitive.

According to Chauhan (1978a), although spine size in the irradiated S. khasianum had an inverse relationship with increasing exposure dose, spines in 10 kR plants were bigger compared to control. In the present study, though the size of spines present on the dorsal surface of S. khasianum leaves was not influenced by radiation, 15 and 20 kR exposures significantly inhibited spine size on the ventral surface. In S. indicum, however, lower exposures (2.5 kR) significantly stimulated size of the spines present on both the surfaces of the sixth leaf. A significant reduction in spine size was observed only on the ventral surface in 20 kR exposure.

Unlike S. khasianum where the leaves of irradiated plants were more spiny, the intensity of spines in S. indicum was less compared to control. This is because in S. indicum though spine number was greatly inhibited by gamma rays, leaf expansion was stimulated by lower exposures and inhibited very little by higher exposures. Unlike this the increase in spine intensity of S. khasianum leaves given low exposures was because compared to leaf area, less injury was caused to spine production. Thus the response of spine intensity to gamma rays in S. indicum and S. khasianum differed from each other. Variation in the response of two species to gamma rays is reported in literature (Gunckel and Sparrow, 1961). 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 effects of a growth hormone in different plants." The differential effects of radiations on different plants can be explained on the above lines since radiations are known to cause hormonal disbalance in irradiated plant-material (Siders et al., 1969; Skoog, 1934, 1935).

The number of berries produced per plant in irradiated S. khasi plants did not appreciably differ from control. On the other hand in S. indicum all gamma-ray exposures caused a significant reduction in berry number and the differences were significant in 10, 15 and 20 kR exposures. More than 50% reduction in berry number per plant was observed in 15 kR plants. Thus unlike S. khasianum berry number was more radiosensitive in S. indicum.

In S. khasianum gamma-ray exposures had no marked effect on berry diameter and volume except 20 kR exposure which reduced both these aspects. Unlike this, in S. indicum though 2.5 kR exposure of gamma rays did not influence berry diameter and volume, the other exposures (5-20 kR) were inhibitory. Further, while in S. khasianum gamma-ray exposures had no effect on seed number per berry, in S. indicum it was decreased.

Berry weight in both the species was reduced by gamma-ray exposures. However, while in S. khasianum only 20 kR exposure could cause significant inhibition, in S. indicum gamma-ray exposures from 10 kR onwards were inhibitory. Thus berry weight in *S. indicum* is more radiosensitive than *S. khasianum*. Radiation-induced reduction in berry weight in *S. khasianum* is reported (Chauhan, 1978a).

Berry yield in irradiated *S. khasianum* plants did not differ significantly from control. In contrast, compared to control irradiated *S. indicum* plants had lesser berry yield. Thus unlike *S. khasianum* berry yield was more radiosensitive in *S. indicum*. Killion *et al.* (1971) reported greater sensitivity of yield compared to stem length and observed that plant radiosensitivity increases once the plants develop reproductive capabilities. The present findings in *S. indicum* provide an evidence in this direction. Gamma radiation is known to decrease yield (Davies, 1968; Kahan, 1969; Killion *et al.*, 1971; Davies, 1973; Davies and Mackay, 1973; Ashraf *et al.*, 1975; Fautrier, 1976; Iqbal, 1980).

The glycoalkaloid content in 20 kR irradiated *S. khasianum* plants was more, compared to control. Though 2.5 kR exposure induced an increase in glycoalkaloid content of *S. indicum*, the higher exposures were inhibitory. Gamma radiation-induced increase in active principle content is known for *Nicotiana* (Kuzin *et al.*, 1963), *Datura* (Kaul, *et al.*, 1973), *Papaver somniferum* (Malik *et al.*, 1977), *Turnera ulmifolia* (Tarar and Dnyansagar, 1977), peppermint (Sadowska, 1979) and *Matricaria recutita* (Selenina and Stepanenko, 1979).

CHAPTER V

SHOOT APICAL MERISTEM

INTRODUCTION

Growth reactions of plants are reflected in the responses of the shoot apex (Gunckel and Sparrow, 1961). A study of the effect of radiations on the shoot apical meristem should, therefore, lead to the understanding of growth responses in the irradiated material. Since no information is available on these aspects in the two species included here (*Solanum khasianum* and *S. indicum*) and as the behaviour of these two species in the radiation experiments described before (Chapters III and IV) were very different, an attempt was made to study radiation effects on the shoot apical meristem and to correlate these with the growth responses of 15 and 60 days old seedlings.

MATERIALS AND METHODS

The methods for the gamma irradiation of seeds are given in Chapter III. When the control and irradiated seeds of the two species began to germinate they were tagged on the day of emergence. For anatomical studies 15 and 60 days old seedlings (from the day of emergence) were fixed in Randolph's modification of Navashin fluid (Johansen, 1940). The shoot apices were dehydrated through tertiary butyl alcohol series, infiltrated and embedded in paraffin (BDH, India). The sections were cut on a rotary microtome at a thickness of 8-10 µm and stained with safranin-Delafield's haematoxylin combination and mounted in D.P.X.

For observations only median longitudinal sections of the shoot apices were considered. Per treatment 5 apices were scored and the characters studied included topography of the apex, cell characteristics of tunica and corpus, cytohistological zonation, distance of cell maturation zone from summit and cell expansion. Cell area was calculated with the help of radial and axial dimensions of the cells. For a particular cell area at least ten cells per apex were measured.

RESULTS

S. khasianum :

(i) Control: The vegetative shoot apex of *S. khasianum* which in the 15 days old seedlings was hemispherical became dome shaped in 60 days old seedlings (Plate 3 a,b). On both the days the tunica was biseriate having dense cytoplasm with almost isodiametric to anticlinally elongated cells. The corpus cells also had dense cytoplasm and were broader than long. Shoot apex organization on both the days exhibited cytohistological zonation pattern (Plate 3 a,b), typical to Angiosperms type (Popham, 1952). Some apices of this species (60 days old) exhibited initiation of flower buds (Plate 3 c). The distance of cell maturation zone from summit increased with increase in age (Table 13). The cells present between 200-300 µm from the summit of the apex in the cell enlargement zone measured 367.87 μm^2 and 192.81 μm^2 in 15 and 60 days old seedlings, respectively (Table 13).

(ii) Irradiated: The apices given various exposures of gamma

Plate 3 (a - l) : Median longitudinal sections of shoot apices of 15 and 60 days old seedlings of different treatments of *S. khasianum* (All figures x 660 except c and e x 320)

a : 15 days old control shoot apex

b : 60 days old control shoot apex

c : Initiation of flowering in 60 days old seedlings of control

d : 15 days old shoot apex given 2.5 kR exposure

e : 15 days old shoot apex given 20 kR exposure

f : 60 days old shoot apex given 5 kR exposure

g : 60 days old shoot apex given 15 kR exposure

h : 60 days old shoot apex given 20 kR exposure

i : Initiation of flowering in 60 days old 20 kR irradiated seedling

j : A part of 20 kR irradiated apex showing cell disruption

k : A part of 20 kR irradiated apex showing tracheidal cells in cortical region

l : A part of 20 kR irradiated apex showing micronuclei

PLATE 3

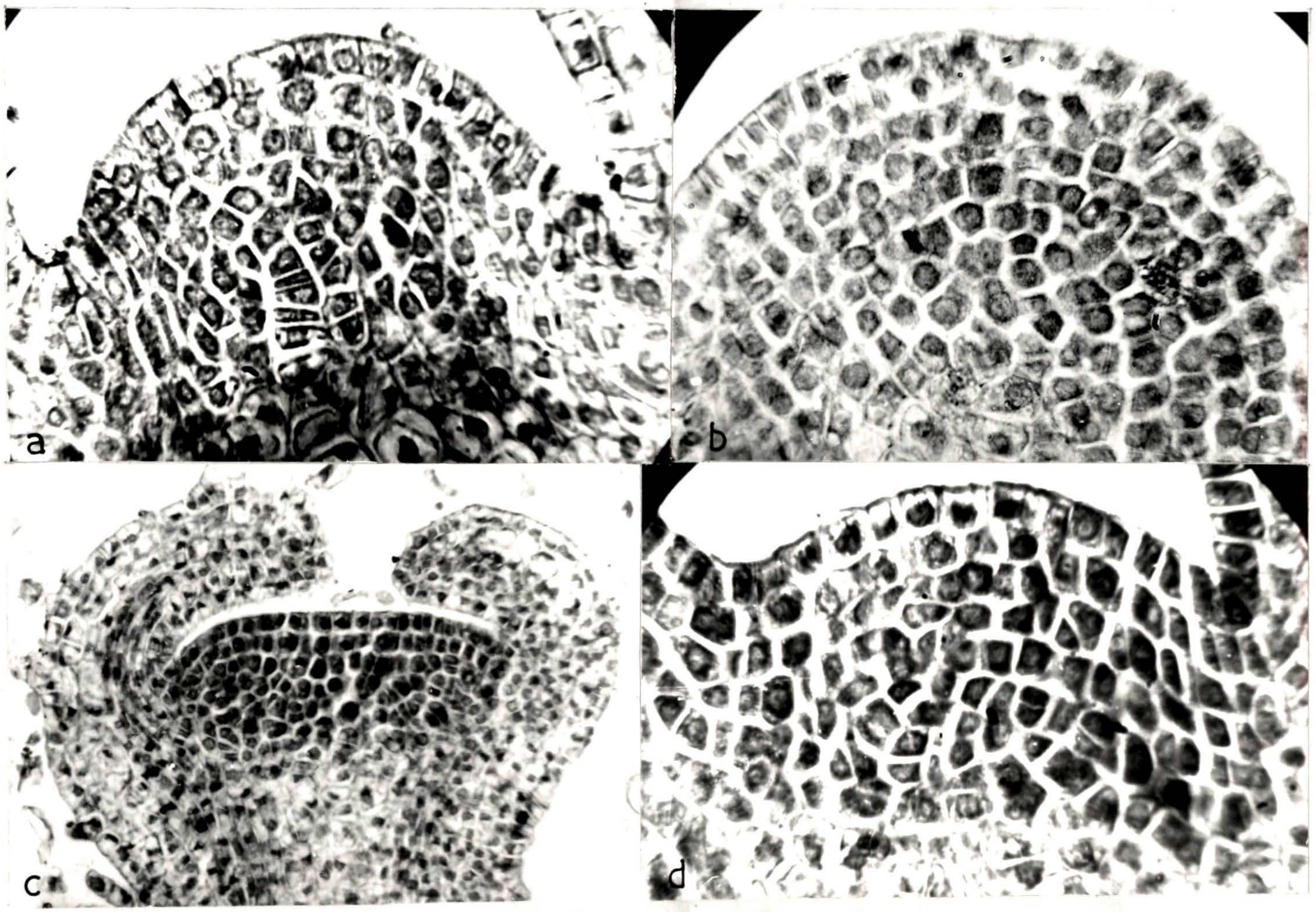


PLATE 3 (Contd)

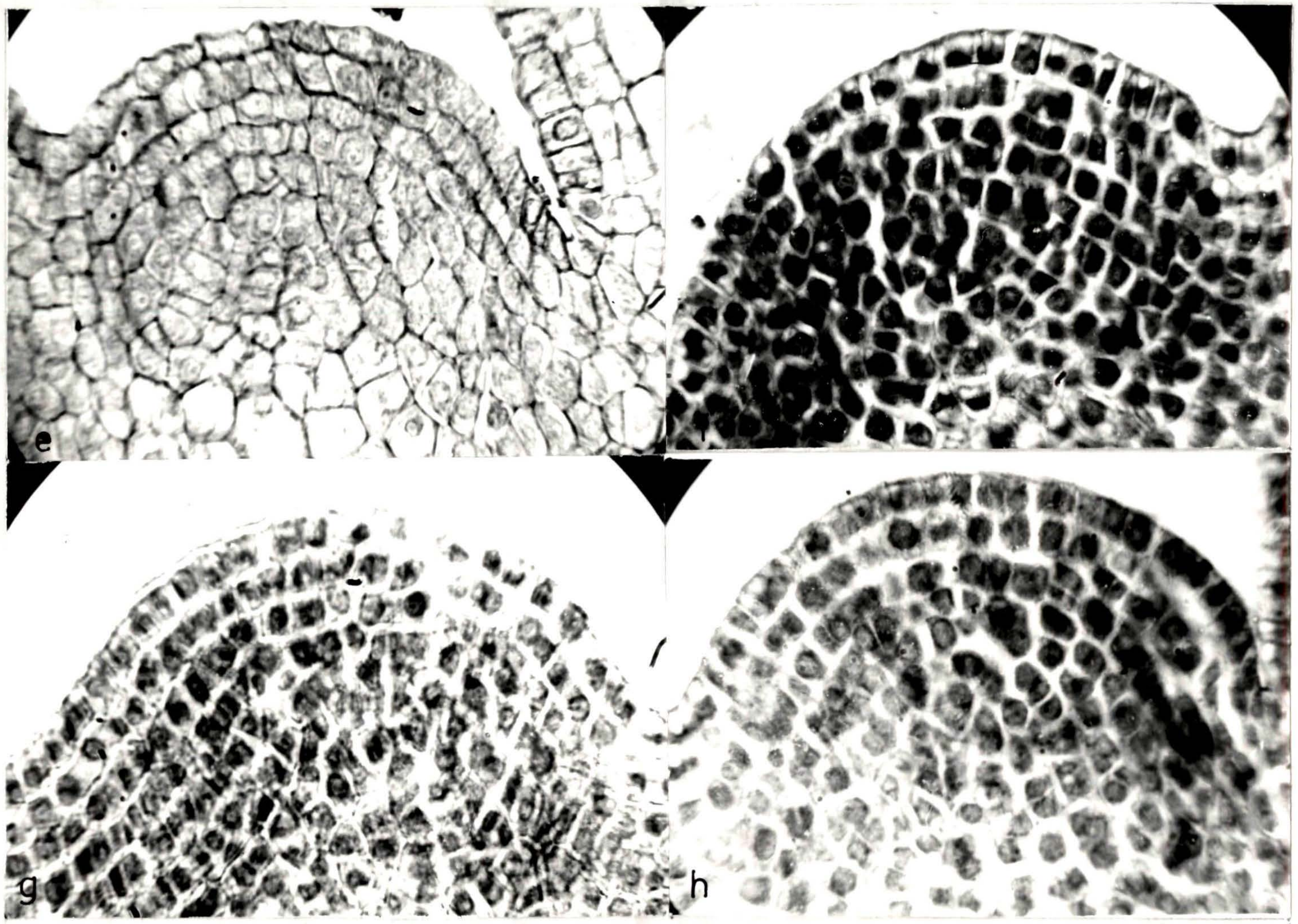


PLATE 3 (Contd.)

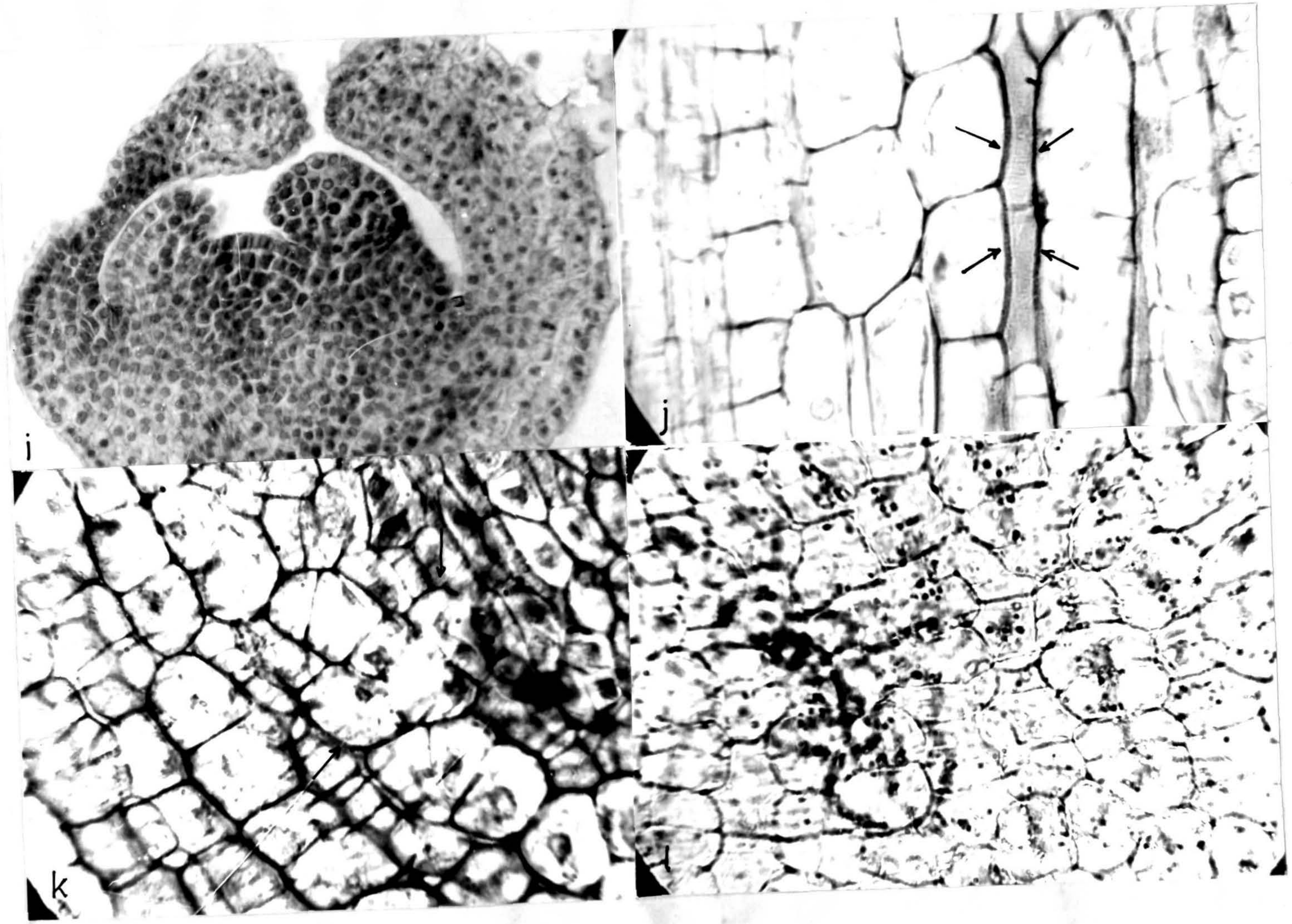


Table 13: Effects of gamma rays on organization of shoot apex in Solanum khasianum

15 DAYS

Treatment	Control	2.5 kR	5.0 kR	10.0 kR	15.0 kR	20.0 kR
Topography of apex	Hemispherical	Hemispherical	Hemispherical	Hemispherical	Hemispherical	Hemispherical
Extent of tunica	2 layered	2 layered	2 layered	2 layered	2 layered	2 layered
Zonation	Present	Present	Present	Present	Present	Present
Cell structure in tunica	Isodiametric to anticlinally elongated dense cytoplasm	Isodiametric to anticlinally elongated dense cytoplasm	Isodiametric to anticlinally elongated dense cytoplasm	Broader than long, little vacuolation	Isodiametric to broader than long, vacuolated cells	Broader than long, vacuolated cells
Cell structure in corpus	Broader than long dense cytoplasm	Broader than long slight vacuolation	Broader than long slight vacuolation	Broader than long slight vacuolation	Isodiametric to broader than long slight vacuolation	Broader than long slight vacuolation
Distance of cell enlargement zone from summit (µm)	78.5	83.0	86.0	83.1	78.0	78.0
Cell area of cells between 200-300 µm (μm^2)	367.87	414.87	335.5	318.75	307.57	298.5
Micronuclei	-	-	-	-	-	-
Cell disruption	-	-	-	-	-	Cell disruption necrosis & tracheary elements in cortical cells
Initiation of inflorescence	-	-	-	-	-	-

Table 13 (Contd.) 60 DAYS

Treatment	Contol	2.5 kR	5.0 kR	10.0 kR	15.0 kR	20.0 kR
Topography of apex	Dome	Hemispherical to dome	Hemispherical to dome	Hemispherical to dome	Hemispherical to dome	Hemispherical to dome
Extent of tunica	2 layered	2 layered	2 layered	2 layered	2 layered	2 layered
Zonation	Present	Present	Present	Present	Present	Present
Cell structure in tunica	Slightly longer than broad, dense cytoplasm	Slightly longer than broad, slightly vacuolated	Longer than broad slightly vacuolated	Isodiametric to longer than broad vacuolated	Longer than broad vacuolated	Longer than broad vacuolated
Cell structure in corpus	Broader than long, dense cytoplasm	Longer than broad, slight vacuolation	Broader than long, slight vacuolation	Isodiametric to broader than long, slight vacuolation	Longer than broad, slight vacuolation	Isodiametric to longer than broad slight vacuolation
Distance of cell enlargement zone from summit (µm)	87.5	93.7	83.1	91.2	87.5	88.3
Cell area of cells between 200-300 µm ($µm^2$)	192.81	193.12	217.87	254.5	237.5	209.79
Micronuclei	-	-	Present	Present	Present	Present
Cell disruption	-	-	-	-	-	-
Initiation of inflorescence	Present	Present	Present	Present	Present	Present

rays in 15 days old seedlings exhibited a hemispherical outline(table 13, Plate 3 d,e). In 60 days old seedlings, however, the shape of the shoot apex in exposed plants exhibited a variation of outline between hemispherical and dome (Plate 3 f-h). The biseriate tunica, which in 15 days old irradiated seedlings had cells broader than long, in 60 days old seedlings was made of cells longer than broad (Plate 3 d-h). Vacuolation of tunica and corpus cells was evident in all the irradiated apices, both on 15 and 60 days (Plate 3 d-h). The irradiated apices in both 15 and 60 days old seedlings exhibited a cytohistological zonation pattern similar to the control apex (Plate 3 d-h). Further, in conformity to the control apex the irradiated apices also exhibited initiation of flowering in 60 days old seedlings(Table 13, Plate 3 i). The distance of cell maturation zone from summit was usually more in 60 days compared to 15 days (Table 13). However, no dose rate relationship could be established. Also, compared to control, the distance was more from the summit of the apex in most of the treatments (Table 13). The cells on the 15th day, in the cell enlargement zone between 200-300 µm from the apex, were larger in 2.5 kR irradiated apices and smaller than control in apices given 5-20 kR exposures (Table 13). On the 60th day, however, the irradiated apices had bigger cells compared to control (Table 13). As in control, apices of 60 days old seedlings had smaller cells in comparison to 15 days old seedlings (Table 13).

Cell disruption and thickened cell walls in the cells of the meristem as also differentiation of tracheidal cells in cortex were observed in 20 kR irradiated apices (Plate 3 j,k).

Micronuclei were witnessed only in apices of 60 days old seedlings given 5-20 kR exposures (Plate 3 l; Table 13).

S. indicum :

(i) Control: The vegetative shoot apices of S. indicum were hemispherical on both the days of scrutiny (Plate 4 a,b). On both the days the tunica was biseriate having dense cytoplasm. The structure of the cells in tunica and corpus exhibited variation from 15 days old seedlings, in 60 days old seedlings (Table 14). Shoot apex organization on both the days exhibited a distinct cytohistological zonation, typical to Angiosperm type (Popham, 1952) (Plate 4 a,b). The distance of cell maturation zone from summit decreased with an increase in age (Table 14). The cells present between 200-300 µm from the summit of the apex in the cell enlargement zone measured 219.37 μm^2 and 195.62 μm^2 in 15 and 60 days old seedlings, respectively (Table 14).

(ii) Irradiated: The apices given various exposures of gamma rays in 15 days old seedlings exhibited a hemispherical outline upto 15 kR (Plate 4 c,d) which almost flattened in 20 kR exposed apices (Plate 4 e). In 60 days old seedlings, however, the shoot apex was hemispherical upto 10 kR and almost flat in 15 and 20 kR irradiated apices (Plate 4 f-h). The tunica which in 15 days old seedlings was biseriate upto 15 kR became uniseriate in 20 kR (Plate 4 c-e). In 60 days old seedlings, however, the

Plate 4 (a - j) : Median longitudinal sections of shoot apices of 15 and 60 days old seedlings of different treatments of *S. indicum* (All figures x 660)

a : 15 days old control shoot apex
b : 60 days old control shoot apex
c : 15 days old shoot apex given 10 kR exposure
d : 15 days old shoot apex given 15 kR exposure
e : 15 days old shoot apex given 20 kR exposure
f : 60 days old shoot apex given 5 kR exposure
g : 60 days old shoot apex given 15 kR exposure
h : 60 days old shoot apex given 20 kR exposure
i : A part of 20 kR irradiated apex showing cell disruption
j : A part of 20 kR irradiated apex showing micronuclei

PLATE 4

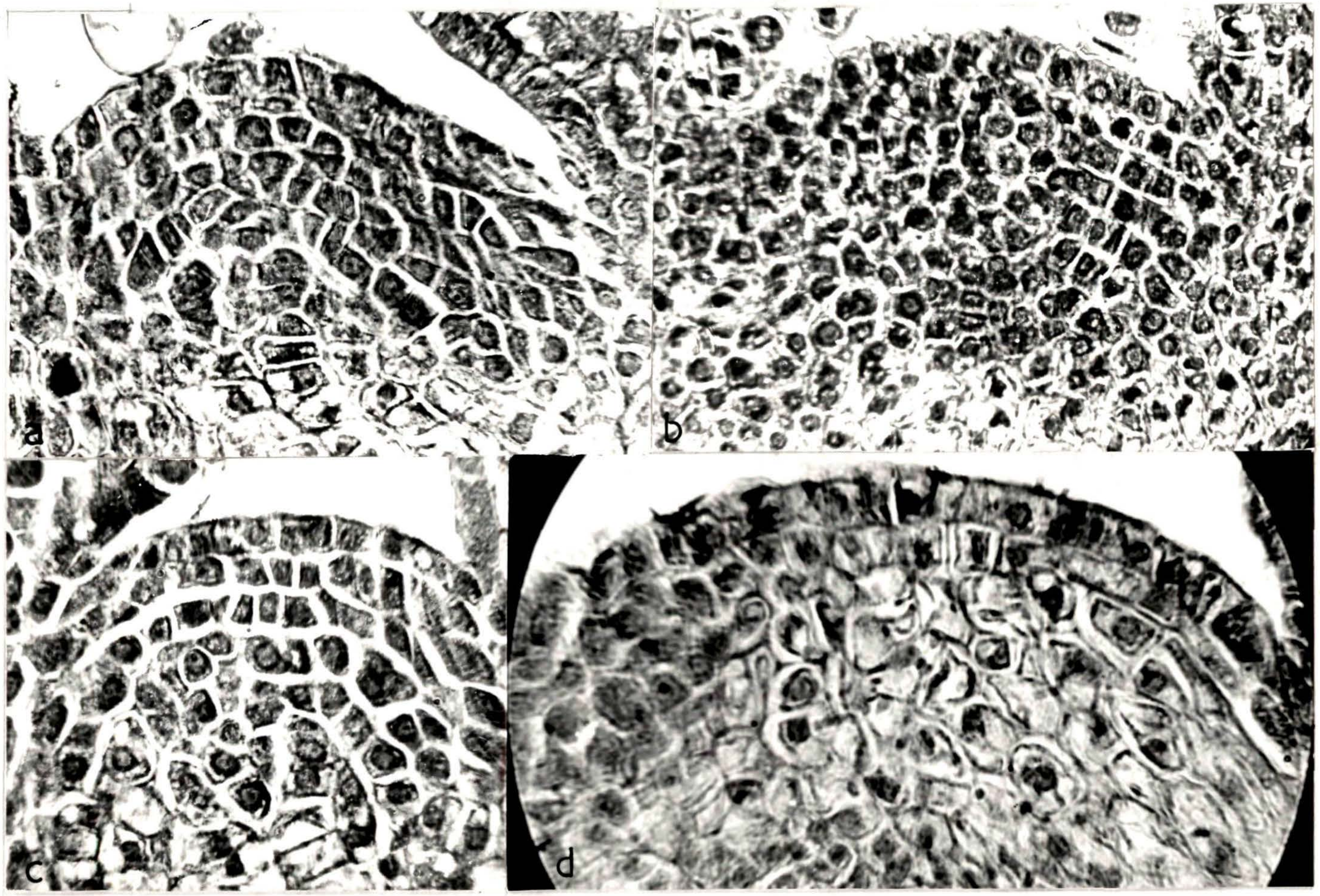


PLATE 4 (Contd)

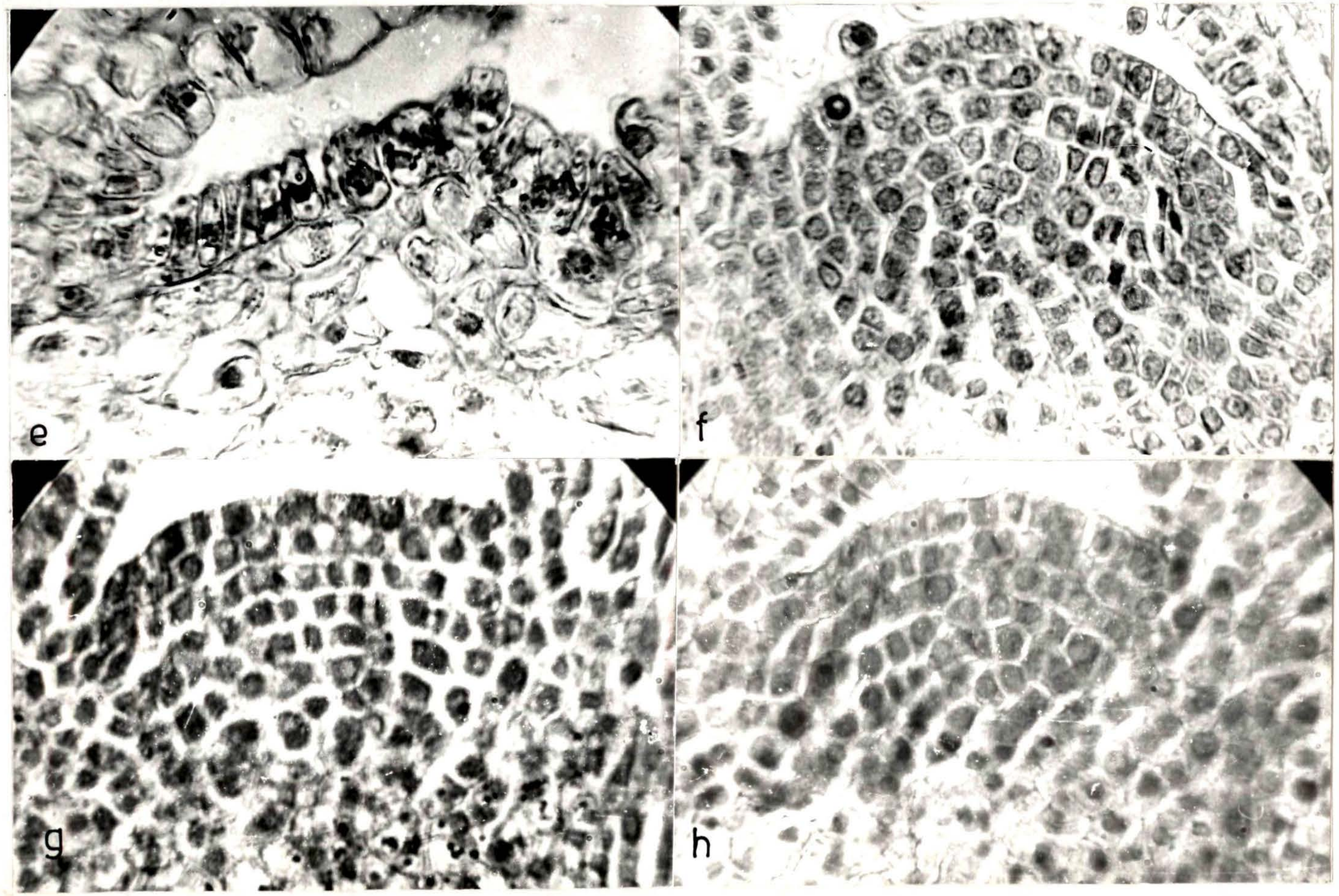


PLATE 4 (Contd)

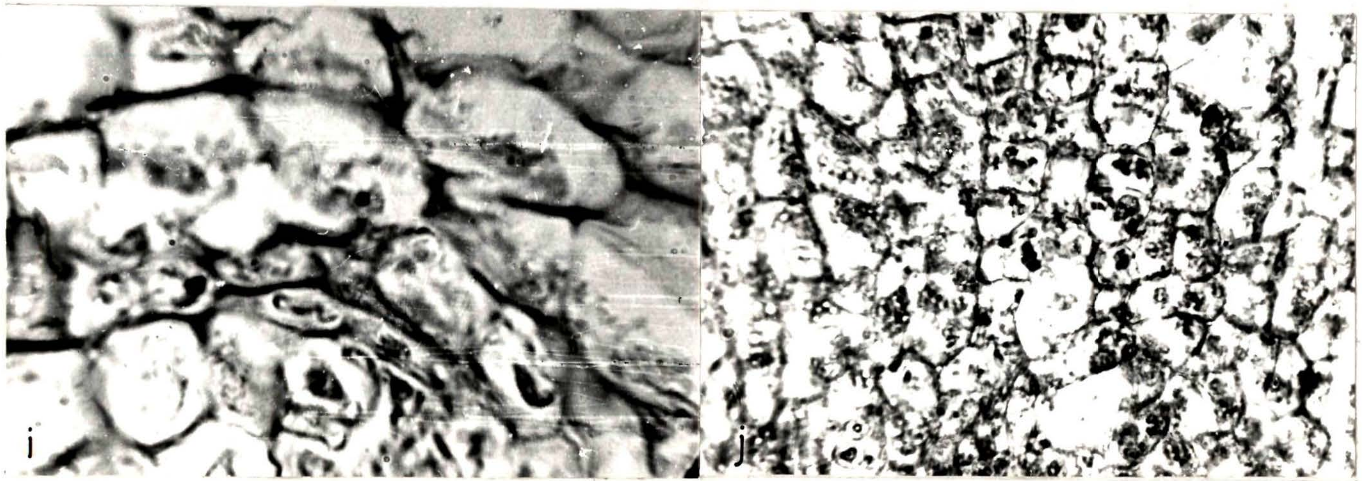


Table 14: Effect of gamma rays on organization of shoot apex in *Solanum indicum*.

15 DAYS

Treatment	Control	2.5 kR	5.0 kR	10.0 kR	15.0 kR	20.0 kR
Topography of apex	Hemispherical	Hemispherical	Hemispherical	Hemispherical	Hemispherical	Hemispherical to flat
Extent of tunica	2 layered	2 layered	2 layered	2 layered	2 layered	Uniseriate
Zonation	Present	Present	Present	Present	Zonation on the verge of dis-organization/ disorganized	Disorganized
Cell structure in tunica	Isodiametric to broader than long, dense cytoplasm	Broader than long, dense cytoplasm	Isodiametric to broader than long, dense cytoplasm	Broader than long, little vacuolated	Broader than long, little vacuolated	Longer than broad highly vacuolated
Cell structure in corpus	Slightly broader than long, dense cytoplasm	Broader than long, dense cytoplasm	Broader than long, dense cytoplasm	Broader than long, slightly vacuolated	Longer than broad, highly vacuolated	Longer than broad highly vacuolated
Distance of cell enlargement zone from summit(um)	81.5	74.0	72.5	70.0	71.2	69.1
Cell area of cells between 200-300 um(um^2)	219.37	278.5	337.0	374.75	420.5	530.42
Micronuclei	-	-	-	-	-	-
Cell disruption	-	-	-	-	-	Cell disruption, necrosis
Initiation of inflorescence	-	-	-	-	-	-

Table 14(contd.) 60 DAYS

Treatment	Control	2.5 kR	5.0 kR	10.0 kR	15.0 kR	20.0 kR
Topography of apex	Hemispherical	Hemispherical	Hemispherical	Hemispherical to nearly flat	Hemispherical to nearly flat	Hemispherical to nearly flat
Extent of tunica	2 layered	2 layered	2 layered	2 layered	2 layered	2 layered
Zonation	Present	Present	Present	Present	Disorganizing & apex stratified	Disorganizing and apex stratified
Cell structure	Broader than long, dense cytoplasm	Isodiametric to slightly longer than broad, slight vacuolation	Isodiametric to slightly longer than broad, slight vacuolation	Isodiametric to slightly longer than broad, slight vacuolation	Isodiametric to slightly longer than broad, slight vacuolation	Isodiametric to slightly longer than broad, slight vacuolation
Cell structure in corpus	Isodiametric to broader than long, dense cytoplasm	Isodiametric to broader than long, slight vacuolation	Isodiametric to broader than long, slight vacuolation	Isodiametric to broader than long, slight vacuolation	Isodiametric to broader than long, vacuolated	Isodiametric to longer than broad, vacuolated
Distance of cell enlargement zone from summit(um)	75.0	74.0	71.8	70.5	63.7	65.5
Cell area of cells between 200-300 um(um^2)	195.62	202.0	251.0	258.0	247.5	272.75
Micronuclei	Absent	Present	Present	Present	Present	Present
Cell disruption	Absent	Absent	Present	Present	Present	Present
Initiation of inflorescence	-	-	-	-	-	-

tunica was biseriate in all the irradiated apices (Plate 4 f-h). In 15 days old irradiated apices the tunica cells were broader than long upto 15 kR (Plate 4 c,d) and prominently longer than broad with highly vacuolated cells in 20 kR (Plate 4 e). In 60 days old seedlings the tunica cells were isodiametric to slightly longer than broad in all the irradiated apices (Plate 4 f-h). Vacuolated tunica cells were observed in all the irradiated apices of 60 days old seedlings (Plate 4 f-h). The corpus cells in 15 days old irradiated apices were similar to control upto 10kR but became much elongated in 15 and 20 kR (Table 14, Plate 4 c-e). The vacuolation was similar to tunica cells. In 60 days old apices the corpus cell structure showed some variation (Table 14, Plate 4 f-h). Vacuolation of corpus cells was evident in all the irradiated treatments (Plate 4 f-h). The irradiated 15 days old apices exhibited a cytohistological zonation upto 10 kR (Plate 4 c); it was on the verge of disorgnization in 15 kR (Plate 4 d) and disorgnized in 20 kR (Plate 4 e). In irradiated 60 days old apices cytohistological zonation was present upto 10 kR (Plate 4 f), and was disorganizing in 15 and 20 kR (Plate 4 g,h). Shoot apices given 15 and 20 kR exposure exhibited stratification (Plate 4 g,h). As in control, the cell maturation zone in 60 days old irradiated seedlings was more proximal to summit than in 15 days old seedlings (Table 14). The cells in the cell enlargement zone between 200-300 µm from the apex were bigger than in control in both 15 days and 60 days old apices. As in control, the apices of 60 days old seedlings had comparatively smaller cells (Table 14).

In 15 days old seedlings whereas cell disorganization was observed only in apices given 20 kR exposure (Table 14, Plate 4 i), in 60 days old seedlings almost all the exposures caused cell disruption (Table 14).

All the irradiated apices of 60 days old seedlings (2.5-20 kR) exhibited micronuclei (Plate 4 j, Table 14).

DISCUSSION

The control apices in both the species had a hemispherical outline in 15 days old seedlings, while 60 days old seedlings revealed a dome shaped apex in *S. khasianum*, it remained hemispherical in *S. indicum*. In irradiated apices the shape of the apex was not much influenced in *S. khasianum* even at 20 kR exposure. However, unlike the control, the topography of the apex in *S. indicum* became nearly flat in plants given 10-20 kR exposures, indicating flattening of the apex. Flattening of the apex is considered a radiation response and is reported for many species (Pratt, 1959, 1963, 1968; Pratt *et al.*, 1959; Bostrack and Sparrow, 1969; Rai and Singh, 1976).

Cells of the tunica and corpus which have dense cytoplasm in control apices on both the days of scrutiny in both the species show vacuolation of the cells in the irradiated apices. Vacuolation was relatively more in *S. indicum* apices. Pronounced vacuolation of the cells in irradiated apices is reported before (Pratt, 1968; Patel and Shah, 1974; Chauhan and Singh, 1975).

An organized and active shoot apex is supposed to have a cytohistological zonation pattern as evident by staining reactions and is correlated with the growth process (Foster, 1938). Both the species studied here reveal Angiosperm type (Popham, 1952) of zonation pattern. Irradiation though could not influence this pattern in *S. khasianum*, the zonation pattern in *S. indicum* exhibited a tendency of disorganization in apices given 15 and 20 kR exposures. The shoot apices exposed to 15 and 20 kR exposures were stratified, also. The disruption and disorganization of cytohistological zonation pattern is considered to be a typical radiation response and is correlated with radiation damage (Patel and Shah, 1974; Chauhan and Singh, 1975; Rai and Singh, 1976). Radiations are known to induce acropetal advancement of cell maturation zone in the radiation injured shoot apices (Bostrack and Sparrow, 1969; Chauhan and Singh, 1975). Unlike *S. khasianum*, where even higher exposures could not induce this response, in *S. indicum* all the irradiated apices, compared to control, had relatively proximal maturation zone. Excessive cell elongation due to radiations are reported for wheat (Foard and Haber, 1961), and *Carthamus tinctorius* (Chauhan, 1976). In the present study while *S. indicum* had consistently larger cells compared to control apices, in *S. khasianum* no definite response could be evinced. Cell disruption and presence of micronuclei are associated with radiation injury in meristems (Clowes, 1964; Pratt, 1968). In the present study while on the 15th day only 20 kR could induce cell disruption, on 60th day almost all the exposures caused cell disruption and revealed micronuclei in *S. indicum*. In *S. khasianum*, on the other hand even though cell disruption was observed in 20 kR irradiated apices of 15 days old seedlings, no cell disruption was evident in irradiated apices of 60 days old seedlings, suggesting recovery. Micronuclei were present only in apices of 60 days old irradiated seedlings. Further, the incidence of occurrence was less intense in *S. khasianum* than in *S. indicum*. Absence of cytohistological zonation, cell disruption, occurrence of micronuclei, increased vacuolation, excessive cell elongation, acropetal advancement of cell maturation zone and flattening of apex are considered to be reflections of inhibited meristematic activity (Pratt, 1968; Rai and Singh, 1976). Since these aspects are more pronounced in *S. indicum* apices vis-a-vis *S. khasianum* it may be assumed that *S. indicum* is more radiosensitive than *S. khasianum*. That it is so, is also reflected in the growth stimulation/inhibition induced by radiation exposures in 15 and 60 days old seedlings (Fig. 6,7). The present study also provides evidence to suggest that the growth reactions of plants are reflected in responses of the shoot apex. Similar were the observations of Chauhan (in press).

Fig. 6: Effect of gamma rays on plant height in *S. khasianum* seedlings.

Fig. 6

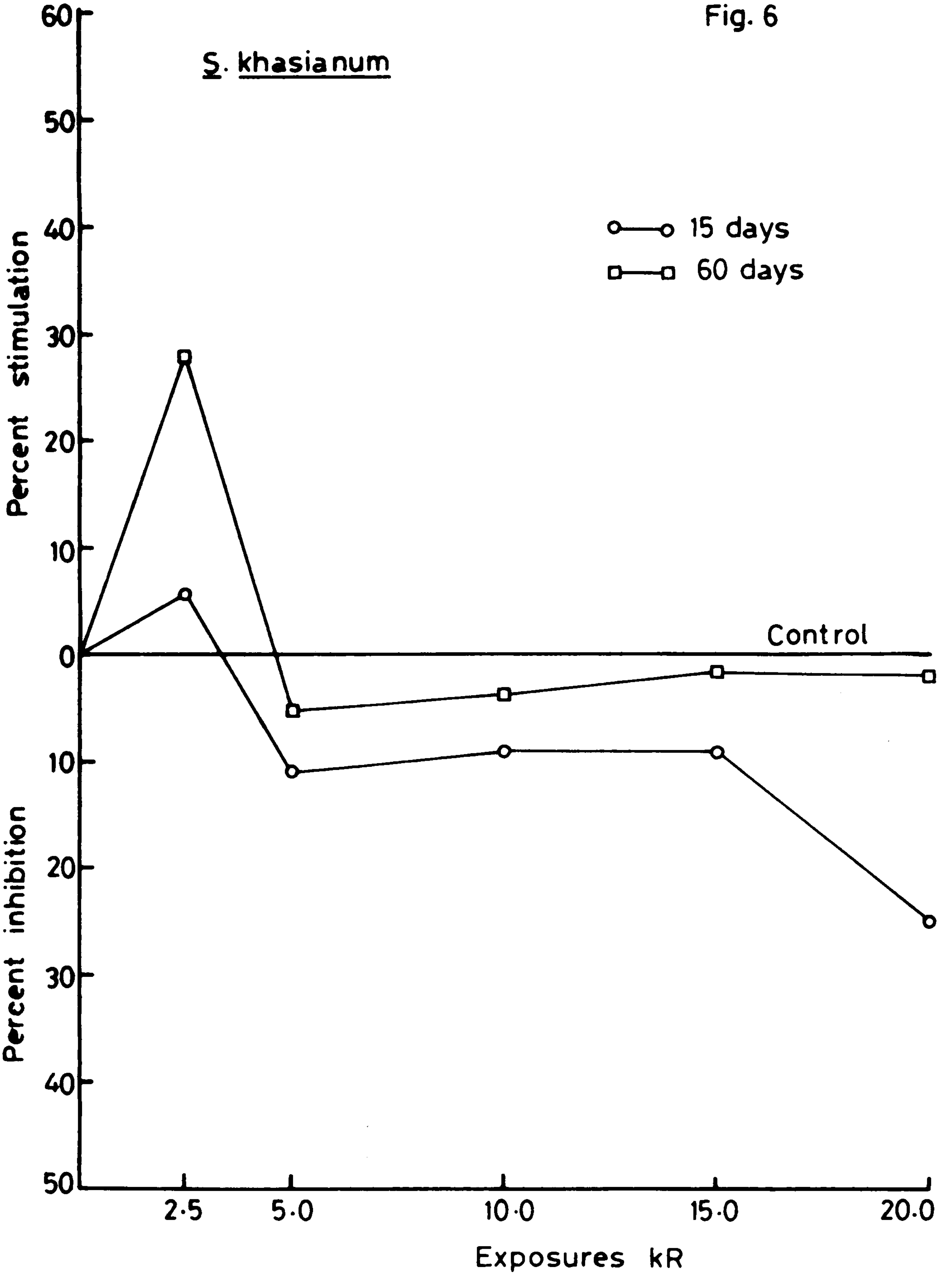
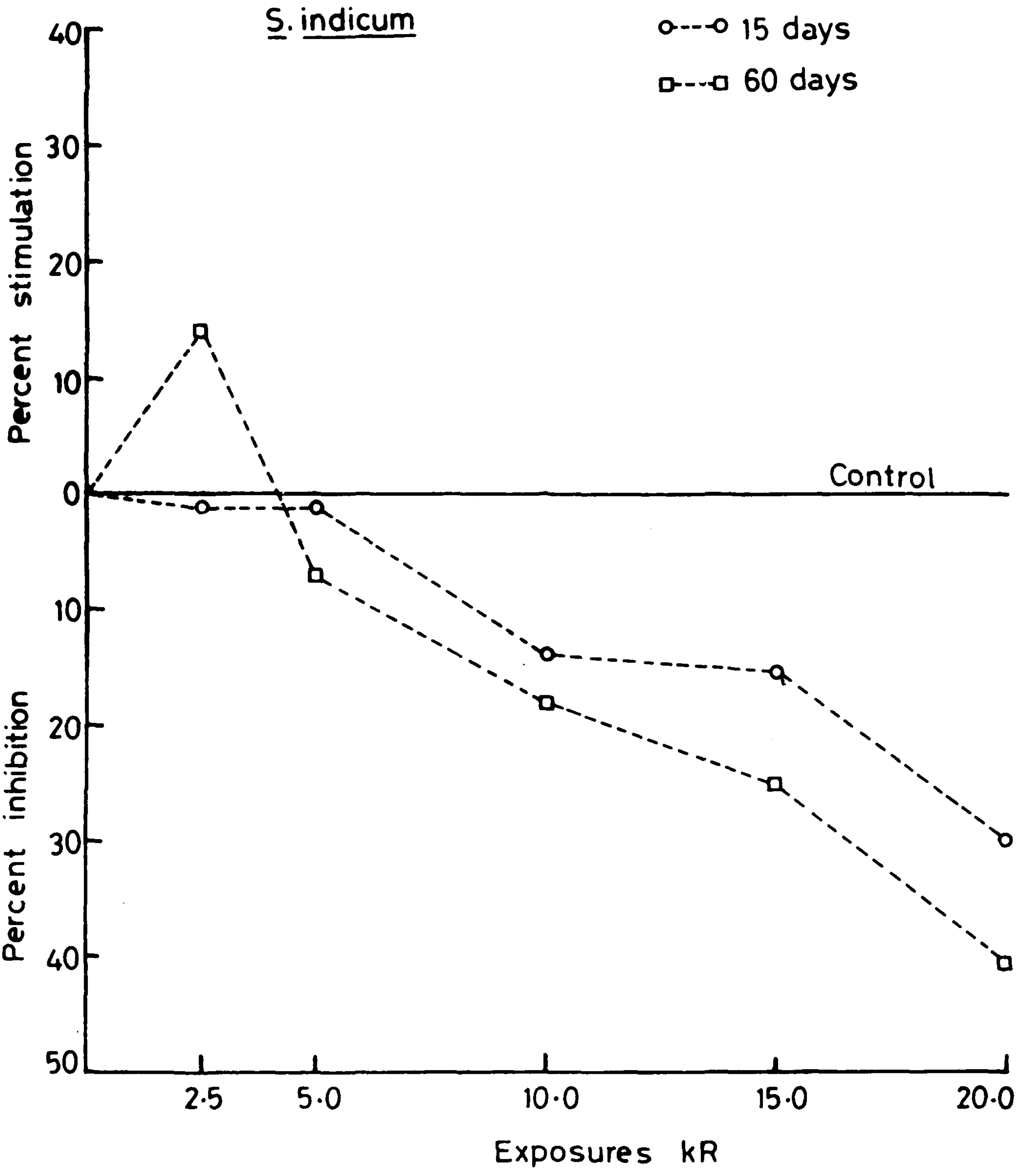

S. khasianum
15 days
60 days
Control
Percent stimulation
Percent inhibition
60
50
40
30
20
10
0
10
20
30
40
50
2·5
5·0
10·0
15·0
20·0
Exposures kR

Fig. 7: Effect of gamma rays on plant height in *S. indicum* seedlings.

Fig. 7


S. indicum
15 days
60 days
Percent stimulation
Percent inhibition
40
30
20
10
0
10
20
30
40
50
Control
2·5
5·0
10·0
15·0
20·0
Exposures kR

CHAPTER VI

FLOWERING RESPONSES

INTRODUCTION

Radiations exert profound influence on the flowering responses of plants. Since information on the flowering behaviour of irradiated *Solanum khasianum* and *S. indicum* plants is lacking an attempt has been made to evaluate the effect of gamma rays on flowering responses (bud emergence, flower production, anthesis, inflorescence (flower cluster) development and berry (fruit) maturity of these two species.

MATERIALS AND METHODS

The number of days after seed sowing for the bud emergence, first anthesis and berry maturity as denoted by yellowing of berries in *S. khasianum* and reddening of berries in *S. indicum* were recorded from control and plants raised from irradiated seeds. Details of seed irradiation and field planting are given in Chapters III and IV. The plants began to flower from July onwards. The number of inflorescences, flower number and number of open flowers per plant were recorded every month from July to October from 10 plants per treatment. Subsequent to October, since only sporadic flowers formed, no data were recorded in November.

RESULTS

S. khasianum :

In *S. khasianum* the onset of reproductive phase is evident with the appearance of first bud 91 days after seed sowing (Table 15). The anthesis of first bud occurred on 123rd day after seed sowing (Table 15). The low exposures of gamma rays (2.5 - 10 kR) did not influence either the process of bud appearance or anthesis, however, both these processes were inhibited by the higher exposures (15 and 20 kR) (Table 15).

The monthly production of inflorescences per plant in control and gamma irradiated plants of S. khasianum are represented in table 16 and figure 8. But for the first month of flowering when irradiated plants (10 kR) produced significantly more inflorescences relative to control, in the later phase of flowering radiation induced inhibition. However, the inhibition caused by any particular exposure could be significant/insignificant at varying time. Further, no relationship with the dose rate could be witnessed. Thus gamma rays did not elicit any definite response on inflorescence production in this species.

The monthly production of flowers per plant in control and gamma irradiated plants of S. khasianum is shown in table 17 and figure 9. A perusal of the figure reveals that though 10 kR exposure increased the number of flowers per plant during early phases of flowering, the flower number was decreased by 15 and 20 kR exposures in August and September, respectively. Towards the close of flowering season in October, but for 5 kR plants which had more flowers compared to control, the control and irradiated plants did not differ significantly (Table 17). Thus gamma-ray exposures upto 10 kR while

Table 15: Effect of gamma rays on bud emergence, anthesis and berry maturity in *Solanum khasianum*.

Treatment	Bud Emergence	First Anthesis	Berry Maturity
CONTROL	91.0 ± 1.3	123.0 ± 0.7	210.0 ± 1.5
2.5 kR	93.0 ± 1.4	125.0 ± 1.2	207.0 ± 1.4
5.0 kR	89.0 ± 0.8	122.0 ± 0.5	205.0* ± 1.4
10.0 kR	94.0 ± 1.1	126.0 ± 1.1	213.0 ± 1.5
15.0 kR	97.0* ± 0.6	129.0* ± 0.5	216.0* ± 1.4
20.0 kR	96.0* ± 1.0	127.0* ± 1.0	216.0* ± 1.2
L.S.D. (p=0.05)	3.5	3.0	4.8

± S.E.

* Significantly different from control (p=0.05)

Table 16: Effect of gamma rays on clusters/plant in *Solanum khasianum*.

Treatment	July	August	September	October
CONTROL	18.4 ± 0.6	26.9 ± 1.9	62.9 ± 8.3	19.7 ± 5.9
2.5 kR	21.6 ± 0.9 (+17.39)	17.2* ± 2.0 (-36.0)	54.7 ± 11.0 (-13.03)	19.4 ± 1.9 (-1.52)
5.0 kR	19.6 ± 1.3 (+6.52)	33.5 ± 2.7 (+24.53)	48.4 ± 10.6 (-23.05)	30.6 ± 6.0 (+55.32)
10.0 kR	23.3* ± 1.4 (+26.63)	26.9 ± 2.5 (0.0)	38.1* ± 5.4 (-39.42)	18.7 ± 1.5 (-5.07)
15.0 kR	20.8 ± 1.8 (+13.04)	13.3* ± 1.1 (-50.55)	57.3 ± 8.8 (-8.90)	18.9 ± 2.5 (-4.06)
20.0 kR	20.0 ± 0.8 (+8.69)	23.4 ± 2.0 (-13.01)	33.8* ± 6.3 (-46.26)	18.5 ± 1.8 (-6.09)
L.S.D. (p=0.05)	3.3	7.2	22.9	

± S.E.

* Significantly different from control (p=0.05)

Figures in bracket represent percent stimulation (+)/inhibition (-) over control

Fig. 8: Effect of gamma rays on number of clusters produced per plant in *S. khasianum*.

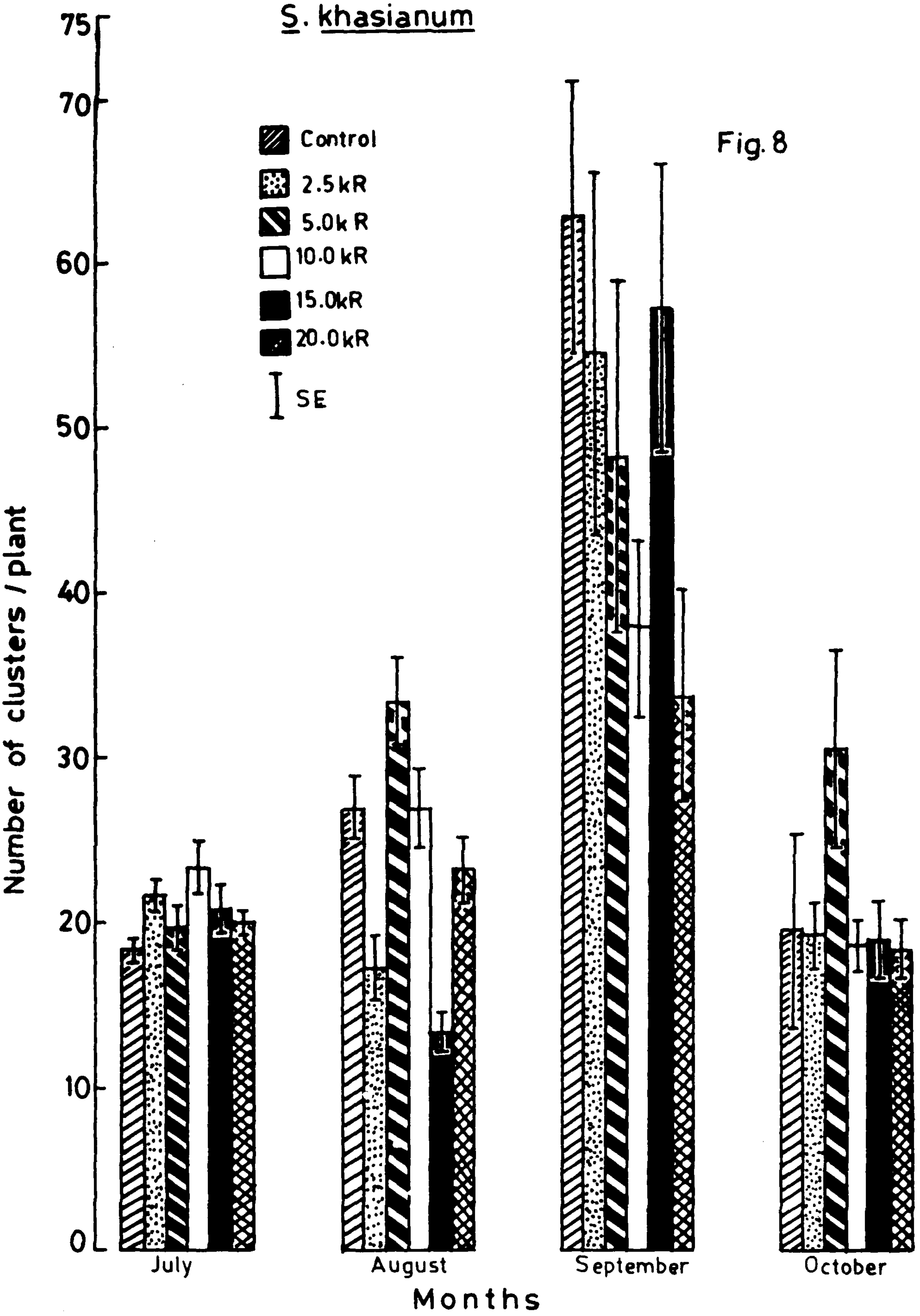


Fig. 8

S. khasianum
Control
2.5 kR
5.0 k R
10.0 kR
15.0 kR
20.0 kR
SE
Number of clusters / plant
0
10
20
30
40
50
60
70
75
July
August
September
October
Months

Table 17: Effect of gamma rays on flower number/plant in Solanum khasianum.

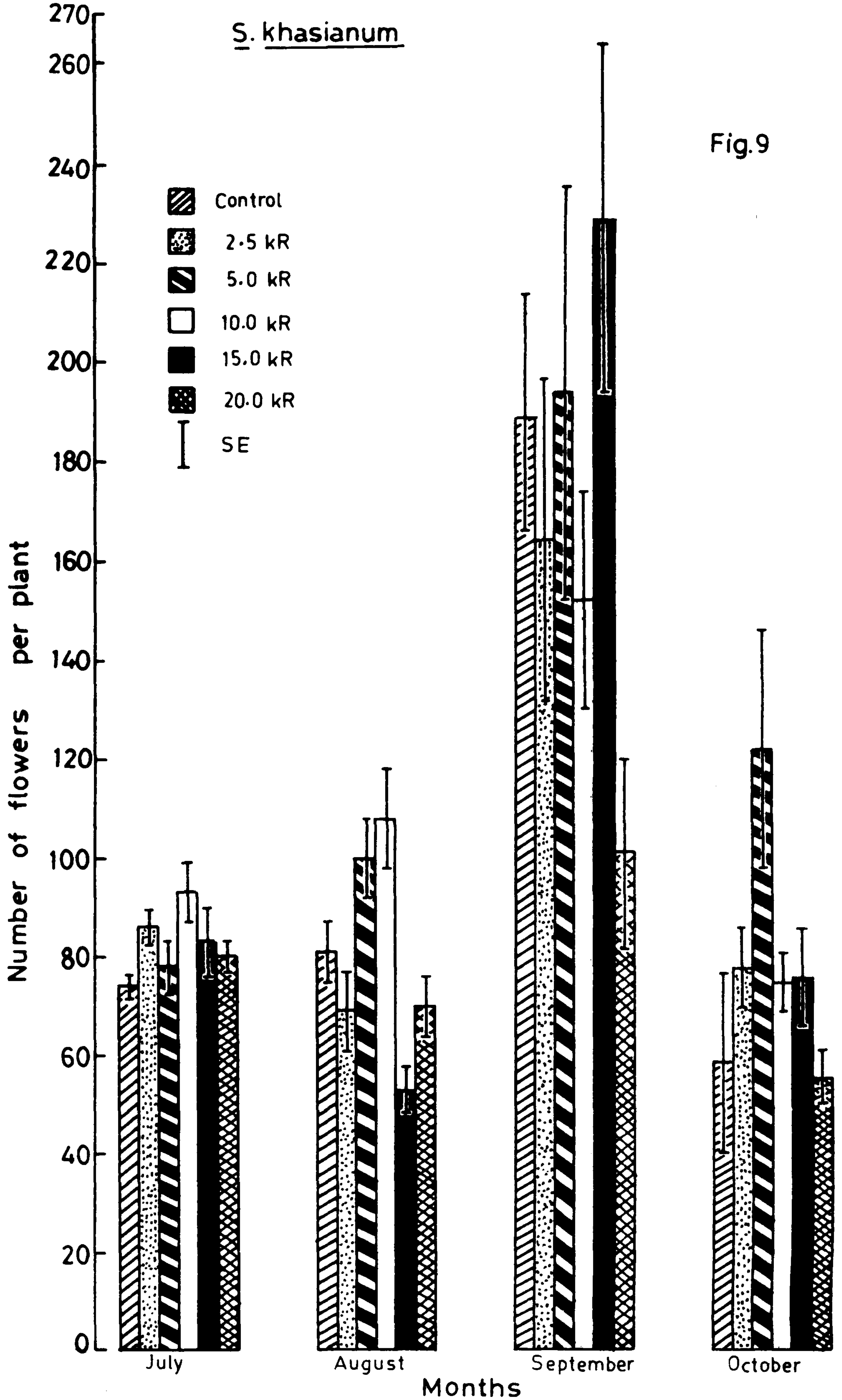
Treatment	July	August	September	October
CONTROL	73.6 ± 2.6	80.7 ± 5.8	188.7 ± 25.1	59.1 ± 17.9
2.5 kR	86.4 ± 3.6 (+17.39)	68.8 ± 8.1 (-14.74)	164.1 ± 33.2 (-13.03)	77.6 ± 7.8 (+31.30)
5.0 kR	78.4 ± 5.3 (+ 6.52)	100.5 ± 8.2 (+24.53)	193.6 ± 42.6 (+ 2.59)	122.4* ± 24.2 (+107.10)
10.0 kR	93.2* ± 5.8 (+26.63)	107.6* ± 10.1 (+33.33)	152.4 ± 21.9 (-19.23)	74.8 ± 6.1 (+26.56)
15.0 kR	83.2 ± 7.3 (+13.04)	53.2* ± 4.6 (-34.07)	229.2 ± 35.3 (+21.46)	75.6 ± 10.0 (+27.91)
20.0 kR	80.0 ± 3.2 (+ 8.69)	70.2 ± 6.2 (-13.01)	101.4* ± 18.9 (-46.26)	55.5 ± 5.6 (-6.09)
L.S.D. (p=0.05)	13.3	25.3	81.1	43.2

± S.E.

* Significantly different from control (p=0.05)

Figures in bracket represent percent stimulation (+)/inhibition (-) over control

Fig. 9: Effect of gamma rays on number of flowers produced per plant in *S. khasianum*.


S. khasianum
Fig.9
Control
2·5 kR
5.0 kR
10.0 kR
15.0 kR
20·0 kR
SE
Number of flowers per plant
270
260
240
220
200
180
160
140
120
100
80
60
40
20
0
July
August
September
October
Months

stimulated flowering at one stage or other, exposures above 10 kR induced inhibition.

The number of open flowers per plant in different months in control and gamma irradiated plants of S. khasianum are given in table 18 and figure 10. It is evident from the figure that at the onset of flowering in July, while 2.5 and 5 kR plants had more open flowers, 20 kR plants had less open flowers, compared to control. In August only 5 kR plants exhibited more open flowers, the number of open flowers per plant, however, was decreased by 10, 15 and 20 kR exposures of gamma rays (Fig. 10). In September also the number of open flowers per plant was maximum in 5 kR plants and minimum in 20 kR plants (Fig. 10). But at the end of flowering season all the gamma irradiated plants had lesser number of open flowers per plant, compared to control(Table 18 Fig. 10). Thus relative to control, low exposures of radiation stimulated flower anthesis and higher exposures inhibited, except in October. Unlike control, (Plate 5 a) in irradiated plants some abnormal flowers (deformed petals and reduced number of stamen) were observed (Plate 5 b-d), however their frequency was not high.

In control berry maturity occurred 210 days after seed sowing (Table 15). Gamma-ray exposures of 2.5 and 10 kR while did not influence berry maturity, the berries in 5 kR irradiated plants matured earlier than control. The higher exposures of gamma rays (15 and 20 kR) delayed berry maturity vis-a-vis control (Table 15).

Table 18: Effect of gamma-ray exposures on number of open flowers per plant in <u>Solanum khasianum</u>.

Treatment	July	August	September	October
CONTROL	9.3 ± 0.5	23.1 ± 1.1	27.6 ± 3.4	19.8 ± 2.7
2.5 kR	11.5* ± 0.5	20.7 ± 1.6	32.3 ± 2.1	10.2* ± 0.7
5.0 kR	10.9* ± 0.9	28.2* ± 1.6	38.3* ± 3.4	13.9* ± 1.2
10.0 kR	8.4 ± 0.3	18.8* ± 1.3	24.4 ± 1.6	10.9* ± 1.0
15.0 kR	7.8 ± 0.4	13.1* ± 0.8	22.2 ± 0.9	9.2* ± 0.6
20.0 kR	7.1* ± 0.3	14.9* ± 0.9	18.5* ± 1.3	7.8* ± 0.5
L.S.D. (p=0.05)	1.5	4.0	8.0	4.4

± S.E.

* Significantly different from control (p=0.05)

Fig. 10: Effect of gamma rays on number of open flowers per plant in *S. khasianum* and *S. indicum*.

Fig.10

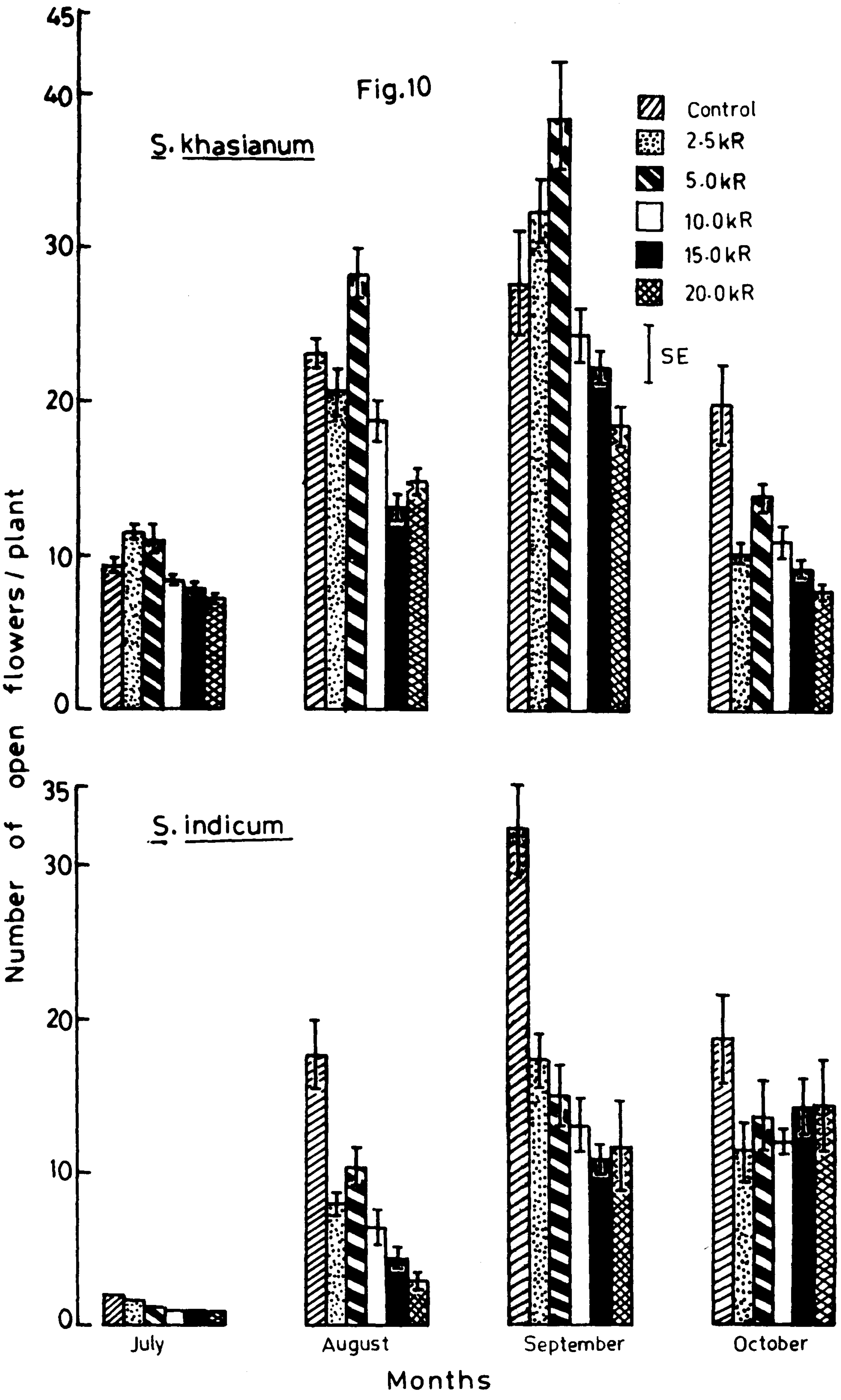
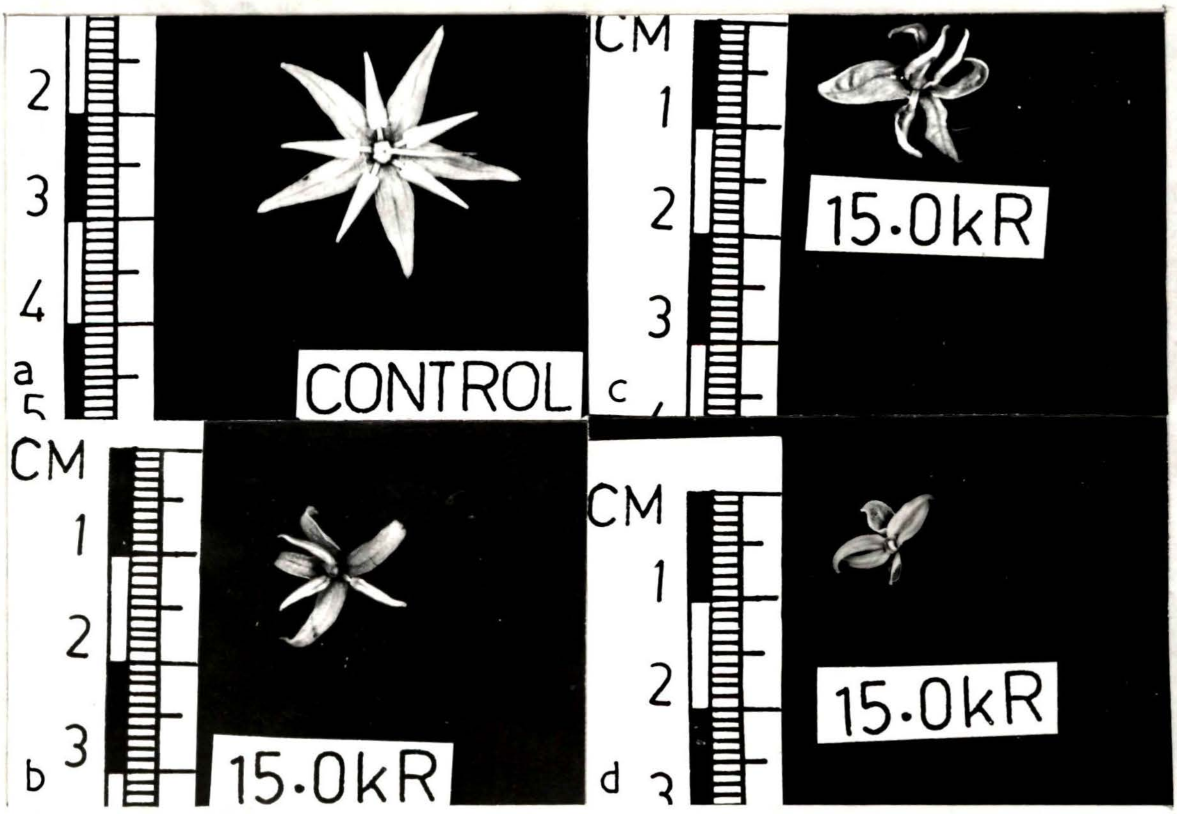

S. khasianum
S. indicum
Control
2.5kR
5.0kR
10.0kR
15.0kR
20.0kR
SE
Number of open flowers / plant
45
40
30
20
10
0
35
30
20
10
0
July
August
September
October
Months

Plate 5 : Flowers produced on control and irradiated plants of
S. khasianum

a : Normal flower

b - d : Flowers exhibiting reduction of petals and stamen

PLATE 5



S. indicum :

S. indicum plants require 116 days for the emergence of flower buds. Compared to control, all the gamma-ray exposures delayed the onset of reproductive phase which became significant in 15 and 20 kR irradiated plants (Table 19). The first bud in control anthesized 139 days after seed sowing (Table 19). Amongst the different gamma-ray treatments, exposures upto 10 kR did not influence anthesis time but higher exposures of gamma rays (15 and 20 kR) delayed first anthesis and the delay was significant vis-a-vis control (Table 19).

The monthly production of inflorescences per plant in control and gamma irradiated plants is represented in table 20 and figure 11. It is clear from the figure that all gamma-ray exposures induced inhibition of inflorescence development which remained significant during most of flowering period, except October.

The figure 12, which shows the average number of flowers produced per plant in control and gamma irradiated plants of S. indicum in different months reveals that all the gamma-ray exposures decreased the number of flowers and the effect persisted for most of the flowering period except October, wherein no appreciable effect of gamma rays on flower development was evident (Table 21, Fig. 12).

The number of open flowers per plant in different months in control and gamma irradiated plants are given in table 22

Table 19: Effect of gamma-rays on bud emergence, anthesis and berry maturity in *Solanum indicum*.

Treatment	Bud emergence	First anthesis	Berry maturity
CONTROL	116.0 ± 1.1	139.0 ± 1.4	204.0 ± 1.3
2.5 kR	119.0 ± 0.7	139.0 ± 2.1	206.0 ± 1.9
5.0 kR	121.0 ± 1.3	144.0 ± 1.3	210.0* ± 0.7
10.0 kR	120.0 ± 1.6	144.0 ± 1.2	210.0* ± 1.2
15.0 kR	124.0* ± 2.5	149.0* ± 2.9	211.0* ± 1.5
20.0 kR	123.0* ± 2.5	147.0* ± 2.0	214.0* ± 2.0
L.S.D. (p=0.05)	5.4	6.2	5.2

± S.E.

* Significantly different from control (p=0.05)

Table 20: Effect of gamma rays on clusters/plant in <u>Solanum indicum</u>.

Treatment	July	August	September	October
CONTROL	10.7 ± 1.5	34.0 ± 5.3	61.8 ± 11.8	60.7 ± 18.5
2.5 kR	11.3 ± 1.2 (+5.60)	11.3* ± 1.3 (-66.76)	27.5* ± 2.8 (-55.50)	50.0 ± 7.4 (-17.62)
5.0 kR	9.6 ± 1.1 (-10.28)	13.6* ± 1.5 (-60.0)	28.3* ± 5.0 (-54.20)	52.9 ± 8.3 (-12.85)
10.0 kR	9.8 ± 1.4 (-8.41)	10.8* ± 1.8 (-68.23)	28.2* ± 5.7 (-54.36)	56.3 ± 12.5 (-7.24)
15.0 kR	8.3 ± 1.4 (-22.42)	5.8* ± 0.8 (-82.94)	21.6* ± 5.4 (-65.04)	50.5 ± 7.9 (-16.80)
20.0 kR	6.4* ± 1.3 (-40.18)	6.2* ± 1.1 (-81.76	26.3* ± 6.2 (-57.44)	49.7 ± 15.7 (-18.12)
L.S.D. (p=0.05)	3.6	8.4	20.3	

± S.E.

* Significantly different from control (p=0.05)

Figures in bracket represent percent stimulation (+)/inhibition (-) over control

Fig. 11: Effect of gamma rays on number of clusters produced per plant in S. *indicum*.

Fig.11

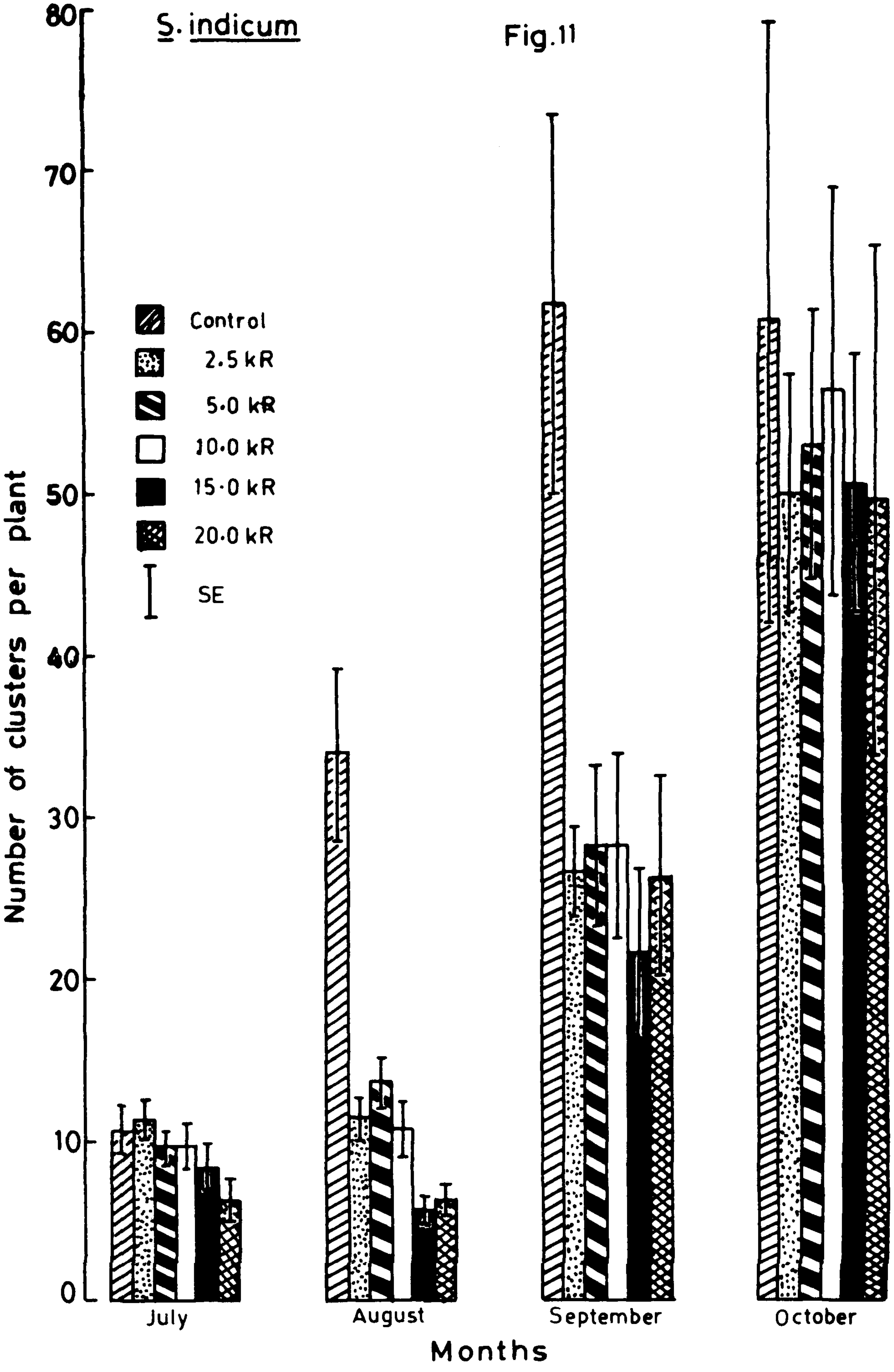

S. indicum
Number of clusters per plant
0
10
20
30
40
50
60
70
80
Control
2.5 kR
5.0 kR
10.0 kR
15.0 kR
20.0 kR
SE
July
August
September
October
Months

Table 21: Effect of gamma rays on flower number/plant in <u>Solanum indicum</u>.

Treatment	July	August	September	October
CONTROL	64.2 ± 9.0	272.0 ± 43.1	370.8 ± 70.8	364.2 ±111.2
2.5 kR	56.5 ± 6.1 (-11.99)	79.1* ± 9.3 (-70.91)	192.5* ± 20.1 (-48.08)	300.0 ± 44.4 (-17.62)
5.0 kR	57.6 ± 7.0 (-10.28)	81.6* ± 9.1 (-70.0)	198.1* ± 35.0 (-46.57)	317.4 ± 49.9 (-12.85)
10.0 kR	58.8 ± 8.5 (-8.41)	64.8* ± 11.1 (-76.17)	225.6* ± 45.9 (-39.15)	337.8 ± 75.1 (- 7.24)
15.0 kR	41.5* ± 7.2 (-35.35)	34.8* ± 5.2 (-87.20)	151.2* ± 38.2 (-59.22)	303.0 ± 47.8 (-16.80)
20.0 kR	32.0* ± 7.0 (-50.15)	31.0* ± 5.5 (-88.60)	210.4* ± 50.2 (-43.25)	347.9 ±109.9 (- 4.72)
L.S.D. (p=0.05)	20.0	63.9	134.9	

± S.E.

* Significantly different from control (p=0.05)

Figures in bracket represent percent (+)/ inhibition (-) over control

Fig. 12: Effect of gamma rays on number of flowers produced per plant in S. indicum.

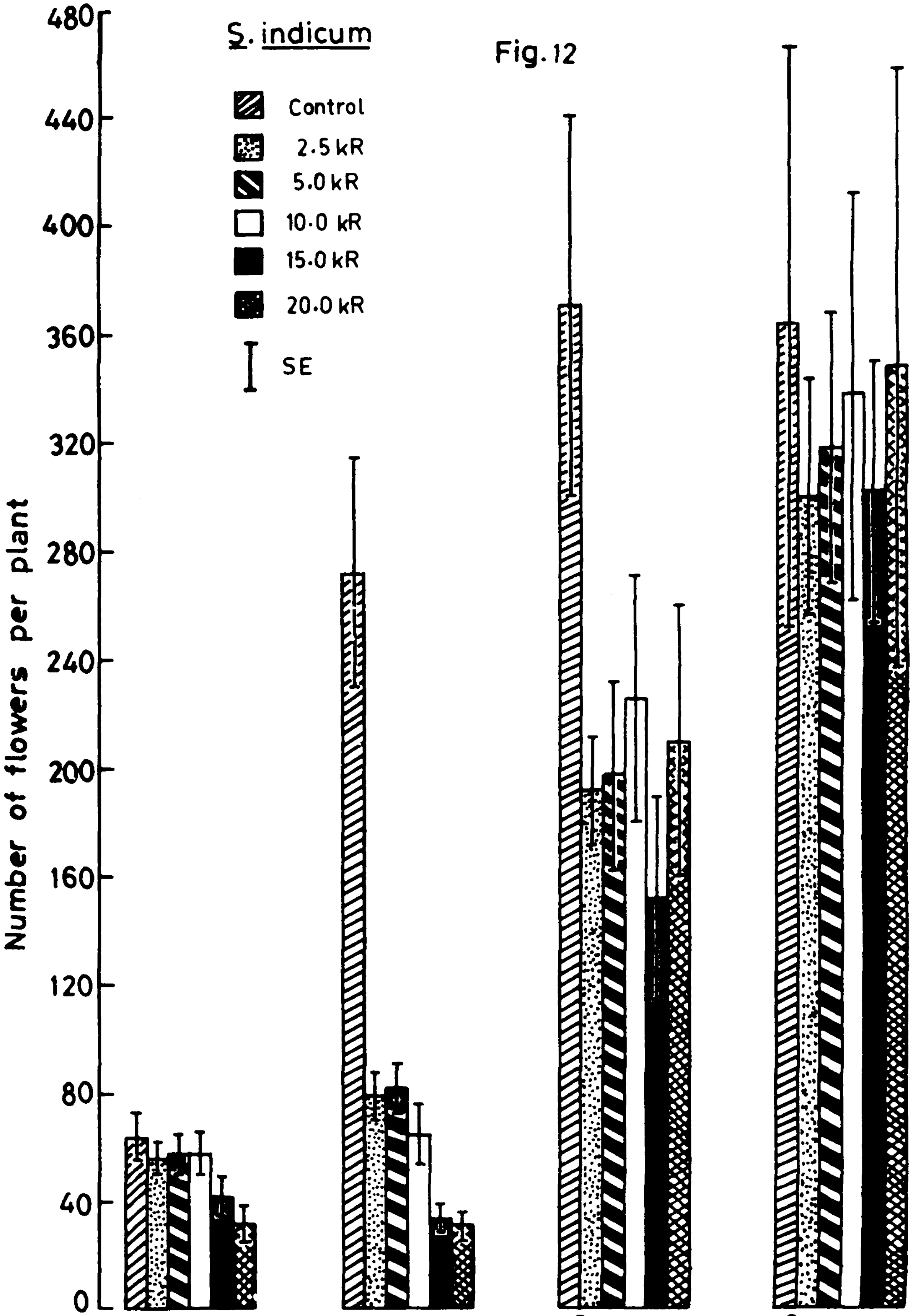

S. indicum
Control
2.5 kR
5.0 kR
10.0 kR
15.0 kR
20.0 kR
SE
Number of flowers per plant
480
440
400
360
320
280
240
200
160
120
80
40
0
July
August
September
October
Months

Fig. 12

Table 22: Effect of gamma-ray exposures on number of open flowers per plant in <u>Solanum indicum</u>.

Treatment	July	August	September	October
CONTROL	1.9	17.7 ± 2.2	32.4 ± 2.9	18.8 ± 3.0
2.5 kR	1.6	7.9* ± 0.7	17.4* ± 1.8	11.3 ± 2.0
5.0 kR	1.2	10.3* ± 1.2	15.0* ± 2.0	13.5 ± 2.3
10.0 kR	1.0	6.4 ± 1.2	12.3 ± 1.7	11.9 ± 0.8
15.0 kR	1.0	4.3* ± 0.6	10.8* ± 1.0	14.2 ± 1.8
20.0 kR	1.0	2.8* ± 0.5	11.8* ± 3.0	14.3 ± 3.1
L.S.D. (p=0.05)		4.1	6.6	

± S.E.

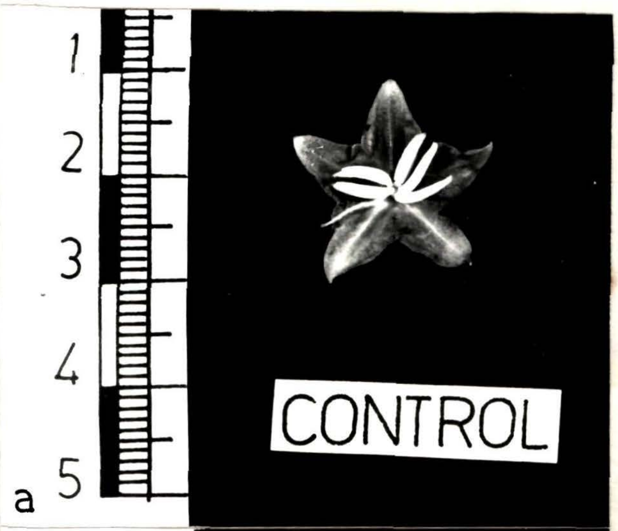
* Significantly different from control (p=0.05)

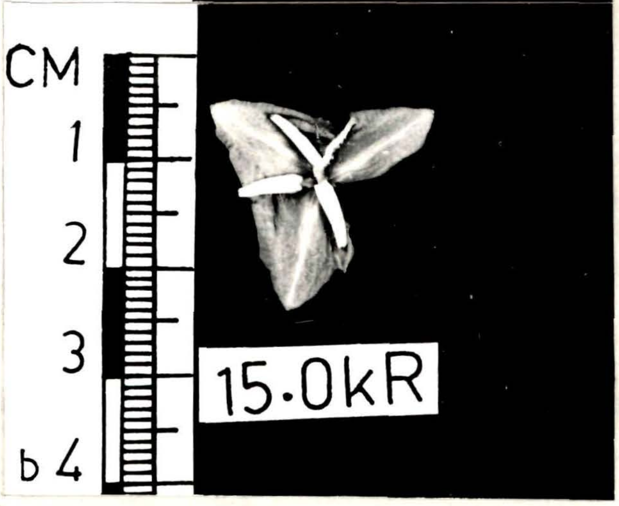
Plate 6 : Flowers produced on control and irradiated plants of *S. indicum*

a : Normal flower

b : Flower showing reduction of petals and stamen

PLATE 6





and figure 10. It is evident from these figures that all gamma-ray exposures inhibited anthesis (Table 22). However, differences were not significant in the first and last month of flowering. Similar to *S. khasianum*, in *S. indicum* also only irradiated plants produced abnormal flowers (reduction of floral parts) (Plate 6 a-b) in very limited numbers.

The maturity of berries occurred on the 204th day after seed sowing and various gamma-ray exposures, except 2.5 kR, significantly delayed the maturity of berries in this species (Table 19).

DISCUSSION

In *S. khasianum* the emergence of first flower buds and its anthesis occurs earlier than *S. indicum*. In both the species while lower exposures of gamma rays did not influence flowering the higher exposures (15 and 20 kR) caused an appreciable delay in flowering. Radiation-induced delay in flowering is known for other plants also (Bowen *et al*., 1962; Gunckel, 1965; Seetharam and Srinivasachar, 1976).

In *S. khasianum* the various gamma-ray exposures did not elicit any definite response in case of inflorescence development and flower formation. In *S. indicum*, however, gamma-ray treatments inhibited inflorescence development and flower formation upto September only. The inflorescence and flower production is more drastically affected by gamma rays in *S. indicum* than in *S. khasianum*. Thus *S. indicum* is more radiosensitive. The fact that in October there was no appreciable difference in the number of inflorescences per plant both in control and gamma irradiated plants of *S. khasianum* and *S. indicum* could be explained on the basis of recovery from radiation damage with progression of time. Recovery from radiation damage is known for other plants also (Gunckel and Sparrow, 1961).

The number of open flowers per plant in *S. khasianum* was significantly increased by low exposures (2.5 and 5 kR) in the early phase of flowering. In the last month of flowering the stimulatory effect was lost and instead inhibition sets in. The plants given 10-20 kR gamma-ray exposures, however, had reduced number of open flowers from the beginning and the inhibition was mostly significant. Kahan (1974), however, reported that in tomato radiation treatments gave significantly more open flowers at the beginning of flowering period. The fact that even stimulatory exposures became inhibitory during the month of October could be the result of onsetting of unconducive environmental conditions. In *S. indicum* the process of anthesis was inhibited by all the gamma-ray exposures which may be a reflection of radiation effects on inflorescence development and flower production in this species.

Berry maturity occurred earlier in *S. indicum* than in *S. khasianum*. In *S. khasianum* 5 kR exposure caused an earliness of berry maturity. Radiation-induced earliness of berry ripening is known for tomato (Kahan *et al.* 1972) and pepper (Maltseva, 1978). Higher exposures of gamma rays delayed fruit ripening in *S. khasianum*. In *S. indicum*, however, berry maturity was significantly delayed by most of the exposures used. Berry maturity in *S. indicum* is influenced more by gamma rays than in *S. khasianum*.

The present study thus brings out the fact that flowering responses of the two species to gamma-ray exposures are different. Variation in the responses of different species are reported (Gunckel and Sparrow, 1961).

CHAPTER VII

POLLEN GERMINATION & POLLEN TUBE GROWTH

INTRODUCTION

Most of the work on radiation effects on in vitro responses of pollen grains pertain mainly to pollen grains irradiated with different types of radiations and their subsequent culturing in nutrient media. However, there are very few reports on the in vitro responses of pollen grains produced in plants raised from gamma-irradiated seeds. Pfahler (1970) has emphasized the importance of such studies. Hence the present investigation was undertaken to study the effect of various gamma-ray exposures (seed irradiation) on the pollen germination and pollen tube growth of *Solanum khasianum* and *S. indicum*, in vitro. Radiations induce hormonal disbalance in the irradiated materials (Skoog, 1934, 1935; Gordon and Weber, 1955; Siders *et al.*, 1969) and exogenous application of hormones can modulate radiation effects (Gaur and Notani, 1960; Chauhan and Singh, 1975; Chauhan, 1976; Bhattacharya, 1977). Since exogenous application of phytohormones is also known to affect pollen germination and pollen tube growth (Johri *et al.*, 1977), an attempt was made to study how gibberellic acid (GA_3), Indole acetic acid (IAA) and 6-furfuryl aminopurine (Kinetin) affect the above radiation responses in the two Solanums included in this investigation.

MATERIALS AND METHODS

I. Effect of gamma rays on pollen germination and pollen tube growth :

The pollen grains of *S. khasianum* and *S. indicum* used in the present study were obtained from plants raised from gamma-irradiated and control (unirradiated) seeds, during 1978. Details of seed irradiation and field planting are given in Chapters III and IV. The pollen grains were germinated on an agarized Brewbaker and Kwack medium (Brewbaker and Kwack, 1964). The composition of the basal medium was 15% sucrose, 100 ppm boric acid, 300 ppm calcium nitrate, 200 ppm magnesium sulphate, 100 ppm potassium nitrate and 0.6% bacto-agar. The media were prepared in double glass distilled water. All the chemicals used in this study were of analar grade (BDH). The pH of the medium was adjusted to 7.3.

The anthers from *S. khasianum* and *S. indicum* plants were collected between 6.00 and 6.30 A.M. The anthers were immediately brought to the laboratory and their tips cut off with a sharp scalpel. The pollen grains were then dusted on a coverslip containing approximately 0.01 ml of the above mentioned medium by gently tapping the anthers. The coverslip was then placed in an inverted position over a metalic ring (thickness 1.5 mm) prefixed to a glass slide and lined with petroleum jelly.

The slides of all treatments were incubated at 30°C for 4 hr at the end of which the germinating pollen grains and pollen tubes were fixed flooding with FAA (5 ml formaldehyde + 5 ml glacial acetic acid + 90 ml 50% ethanol). Four replicate slides were maintained per treatment. The number of germinated and ungerminated grains were counted from 5 microscopic fields chosen randomly per slide. Similarly for the pollen tube growth 25 pollen tubes per slide were selected randomly and measured.

II. Effect of growth hormones and modulation of radiation responses :

Based on the information gathered from the above experiment, in 1979 another experiment was planned to study how various plant growth hormones modulate radiation responses. During the course of this investigation effect of various plant growth hormones on germination and elongation of pollen tube in pollen produced on control (unirradiated) plants was also attempted. The following plant growth hormones were used in this study:

1) Gibberellic acid (GA_3) (Phylaxia, Budapest)
2) Indole acetic acid (IAA) (E. Merck, India)
3) 6-furfurylaminopurine (Kinetin) (Loba-Chemie, Austria).

The concentrations employed were 1, 5, 10 and 25 ppm.

GA_3 and IAA were dissolved first in few drops of ethanol while kinetin was dissolved in few drops of 1N HCl and different concentrations of these hormones were prepared by diluting with the Brewbaker and Kwack basal medium. The pH of the medium was adjusted to 7.3. The liquid media were jelled with 0.6% bacto-agar.

The pollen grains used in this study were obtained from control and 20 kR irradiated plants of S. khasianum and S. indicum. In this experiment pollen from 20 kR irradiated plants were used as the radiation effects were most pronounced in this treatment only. The procedure adopted for setting up this experiment was as outlined above. Three replicate slides were maintained per treatment. The number of germinated and ungerminated grains were counted from 5 microscopic fields chosen randomly per slide and twentyfive pollen tubes selected randomly per slide were measured for pollen tube growth.

RESULTS

A. POLLEN GERMINATION

I. Gamma-ray effects :

S. khasianum :

The pollen produced on control plants exhibited a germination percentage of 56.2% (Table 23). A perusal of table 23 and figure 13 reveals that gamma-ray exposures had no significant influence on pollen germination in this species.

S. indicum :

The pollen collected from control plants exhibited 59.0 percent germination. Gamma-ray exposures of 2.5 and 5.0 kR though did not influence pollen germination, 10-20 kR exposures reduced pollen germination percentage significantly (Table 24, Fig. 13).

II. Effect of growth hormones and modulation of radiation responses :

S. khasianum :

In this experiment 55.1% of the control pollen grains germinated (Table 25). The various hormone treatments viz. GA_3, IAA and kinetin inhibited pollen germination significantly and

Table 23: Effect of gamma rays on pollen germination and pollen tube growth in _Solanum khasianum_.

Treatment	Pollen germination (%)	Pollen tube length' (µm)
CONTROL	56.2 ± 3.7 (49.0)	237 ± 9.1
2.5 kR	50.3 ± 2.3 (45.2) (-10.4)	244 ±10.7 (+2.95)
5.0 kR	51.1 ± 2.7 (45.6) (-9.0)	251 ±10.3 (+5.90)
10.0 kR	54.8 ± 3.5 (47.9) (-2.4)	208 ± 6.8 (-12.23)
15.0 kR	49.9 ± 3.4 (44.9) (-11.2)	162* ± 6.7 (-31.64)
20.0 kR	48.5 ± 1.4 (44.1) (-13.7)	156* ± 6.0 (-34.17)

± S.E.

' Mean of 100 measurements

* Significantly different from control according to student's 't' test.

Figures within parenthesis are transformed means.

- or + denotes percent inhibition (-), stimulation (+) values over control.

Table 24: Effect of gamma rays on pollen germination and pollen tube growth in <u>Solanum indicum</u>.

Treatment	Pollen germination (%)	Pollen tube length• (µm)
CONTROL	59.0 ± 2.4 (50.3)	510 ± 16.6
2.5 kR	55.1 ± 2.0 (47.9) (-6.6)	487 ± 14.9 (-4.50)
5.0 kR	52.4 ± 1.9 (46.3) (-11.1)	481 ± 20.1 (-5.68)
10.0 kR	43.0 ± 2.1 (40.9*) (-27.1)	471 ± 19.0 (-7.64)
15.0 kR	35.4 ± 2.1 (36.3*) (-40.0)	439* ± 14.3 (-13.92)
20.0 kR	24.7 ± 2.4 (30.4*) (-58.1)	398* ± 15.4 (-21.96)
L.S.D. (p=0.05)	4.0	

± S.E.

• Mean of 100 measurements

* Significantly different from control according to student's 't' test.

Figures within parenthesis are transformed means

- or + denotes percent inhibition (-), stimulation (+) values over control

Fig. 13: Effect of gamma rays on pollen germination in *S. khasianum* and *S. indicum*.

Fig. 13

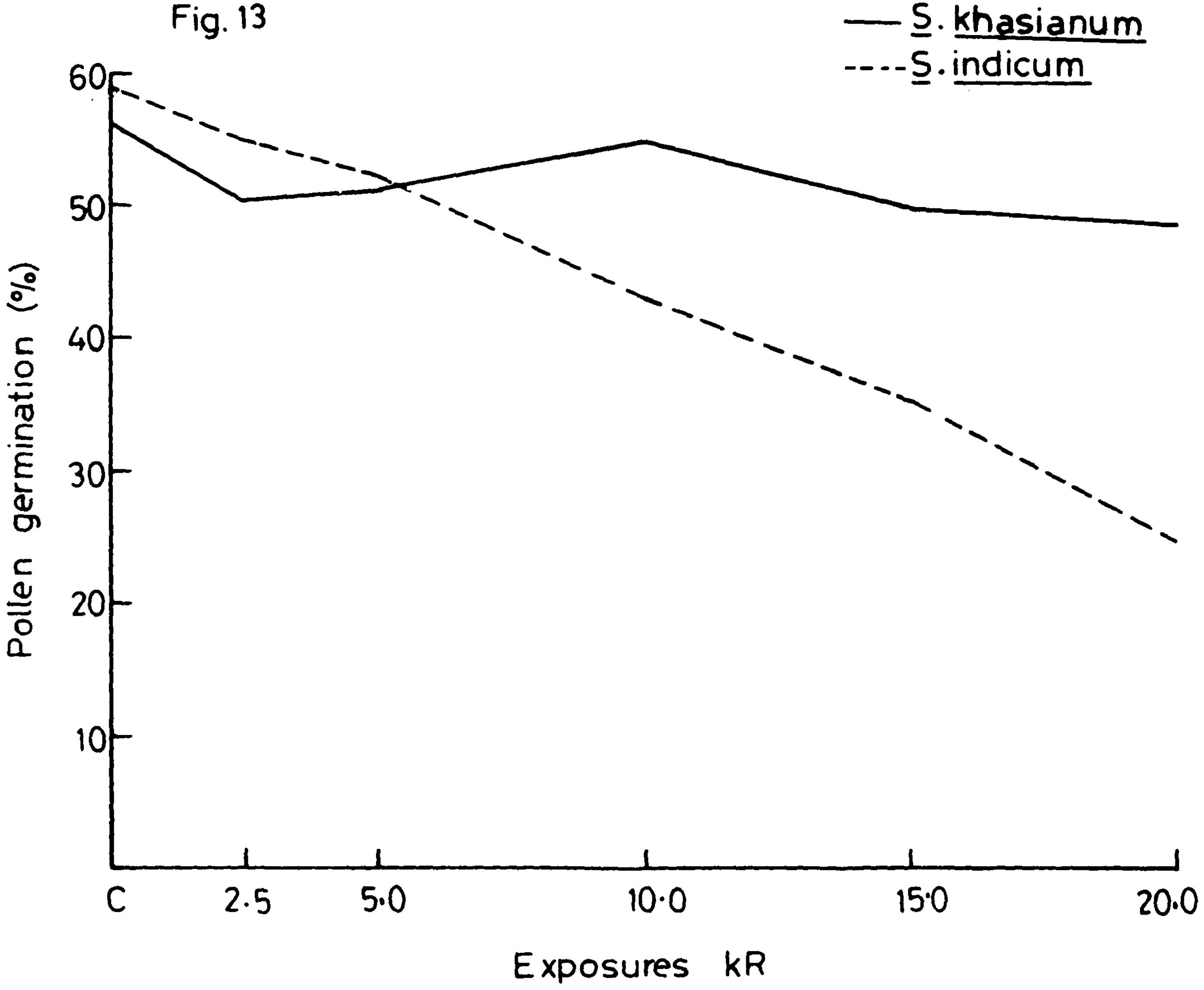

S. khasianum
S. indicum
60
50
40
30
20
10
Pollen germination (%)
C
2·5
5·0
10·0
15·0
20·0
Exposures kR

Fig. 14: Effect of gamma rays on pollen tube growth in *S. khasianum* and *S. indicum*.

Fig.14.

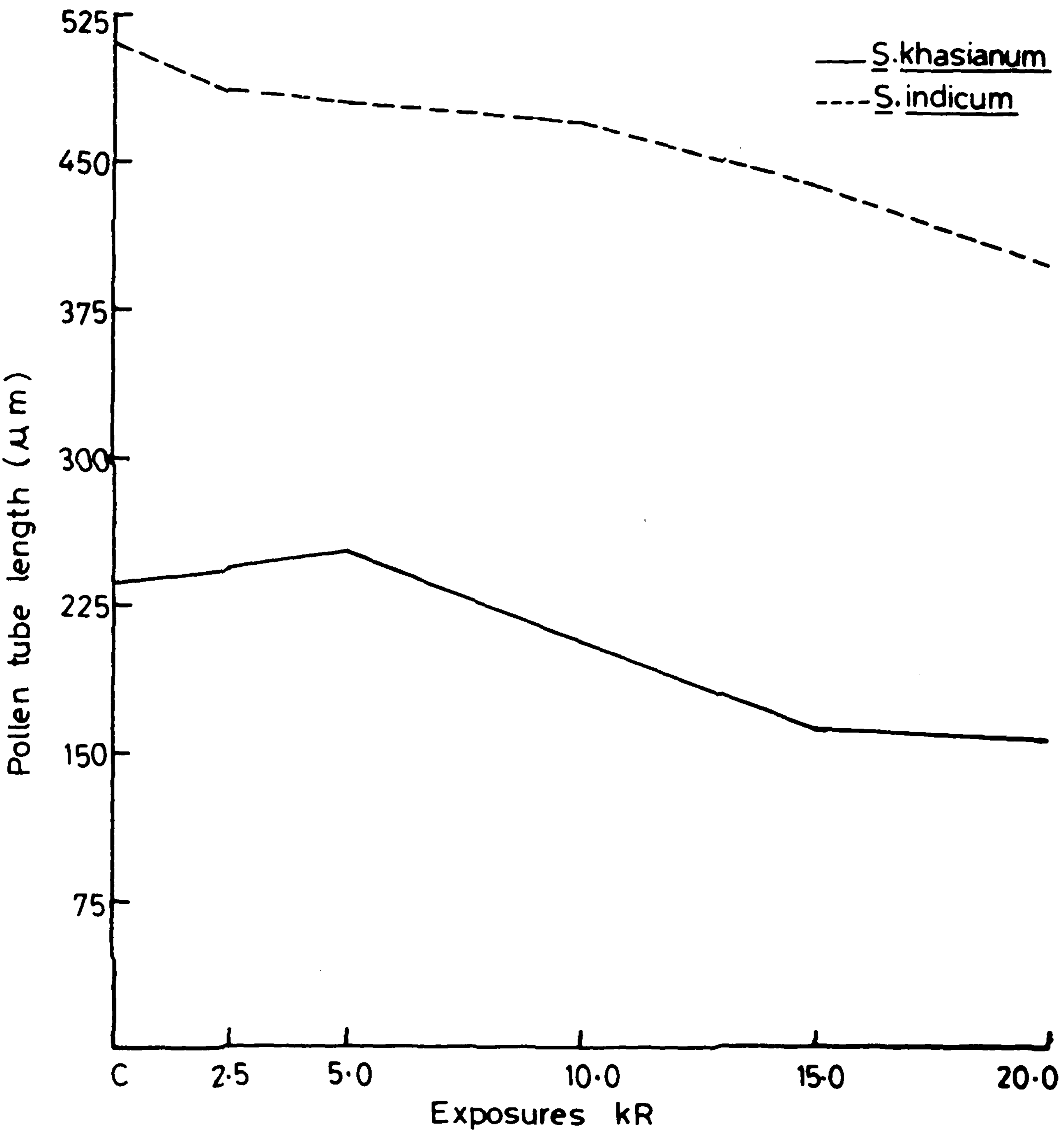
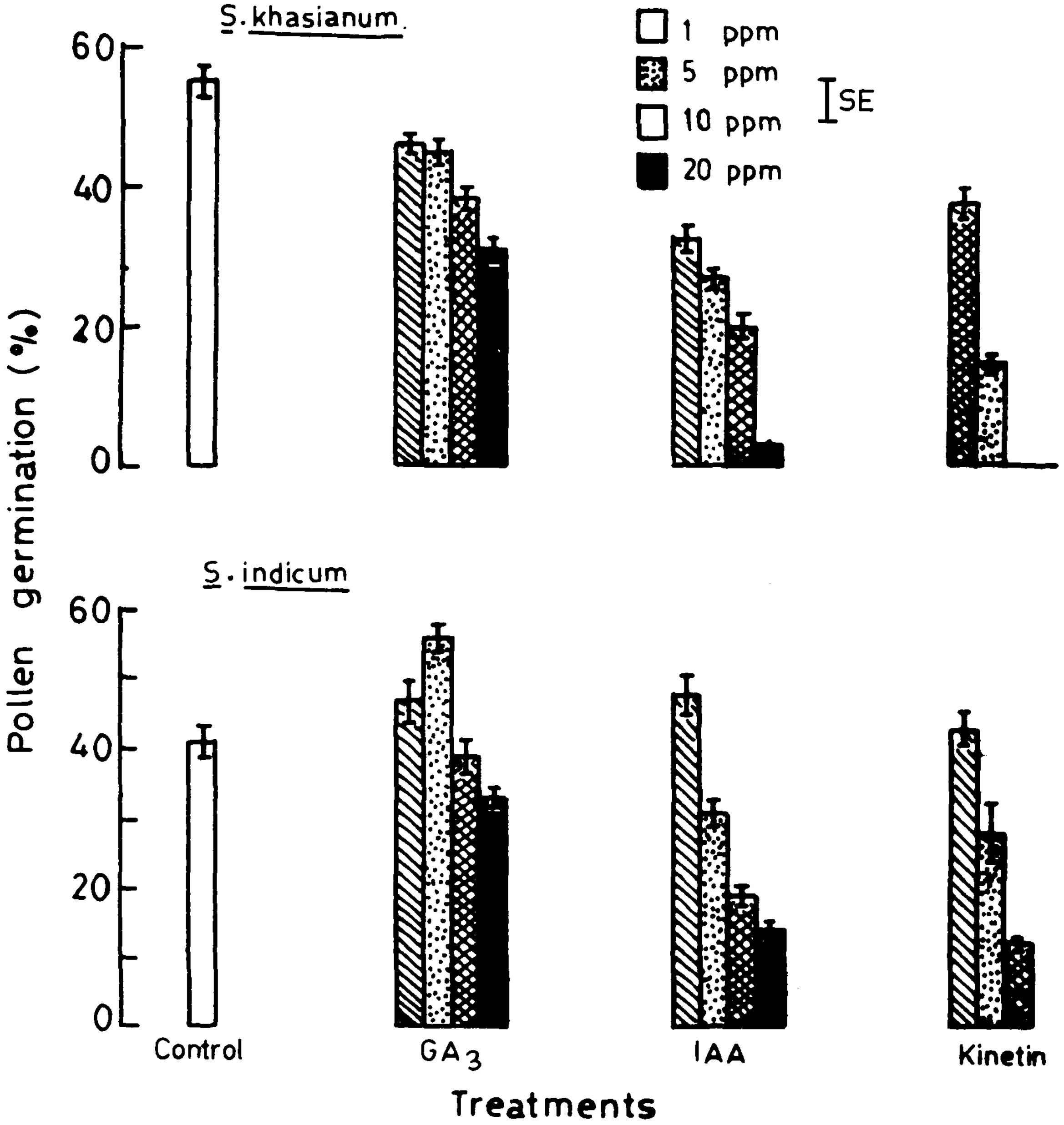

525
450
375
300
225
150
75
Pollen tube length (μm)
C
2·5
5·0
10·0
15·0
20·0
Exposures kR
—— S. khasianum
- - - - S. indicum

Fig. 15: Effect of growth hormones on pollen germination in Solanums.

Fig.15


S.khasianum
S.indicum
1 ppm
5 ppm
10 ppm
20 ppm
SE
Pollen germination (%)
60
40
20
0
Control
GA3
IAA
Kinetin
Treatments

the inhibition increased with increasing concentration (Table 25, Fig. 15).

The pollen grains collected from 20 kR plants exhibited very poor germination, compared to control (Table 25, Fig. 16 and Plate 7 a,b). Effect of interaction of various growth hormones and gamma rays on radiation injury was studied by germinating the pollen grains of 20 kR plants in different growth hormones (GA_3, IAA and kinetin) supplemented media. From the above table and figure it is evident that GA_3 concentrations of 1, 5 and 10 ppm did not alter significantly the germinability of irradiated pollen. Twentyfive ppm GA_3, however, further decreased the germination percentage of irradiated pollen indicating radiosensitization (Table 25, Fig. 16 and Plate 7 c). Similarly, while low concentrations of IAA (1 and 5 ppm) did not affect the germinating capacity of irradiated pollen, the higher concentrations (10 and 25 ppm) further enhanced radiation damage (Table 25, Fig. 16 and Plate 7 d,e). In the kinetin supplemented medium, except one ppm kinetin which did not affect radiation injury to pollen germination the other concentrations (5, 10 and 25 ppm) drastically reduced the germinability of irradiated pollen (Table 25, Fig. 16 and Plate 7 f,g). Thus it becomes evident from the results that GA_3, IAA and kinetin concentrations above a threshold level enhanced the radiation damage to pollen germination in this species.

S. indicum :

The control pollen grains exhibited a germination

Table 25: Effect of growth hormones and modulation of radiation responses of pollen germination (%) in Solanums

Species	Treatment	Control	GA_3				IAA				KINETIN			
			1 ppm	5 ppm	10 ppm	25 ppm	1 ppm	5 ppm	10 ppm	25 ppm	1 ppm	5 ppm	10 ppm	25 ppm
S. khasianum	Control	55.1 ±2.4	45.8* ±1.6	44.7* ±1.9	38.5* ±1.7	30.7* ±1.9	32.9* ±2.2	27.2* ±1.3	20.2* ±1.9	3.2* ±0.2	37.8* ±2.1	15.1* ±1.2	0.0	0.0
	20 kR Irradiated	29.6 ±2.6	32.1 ±1.8	34.5 ±4.0	35.3 ±2.3	8.9** ±1.3	34.6 ±2.4	24.2 ±1.9	16.1** ±0.8	0.0	29.3 ±2.1	5.7** ±0.9	3.4** ±0.4	0.0
S. indicum	Control	41.1 ±2.3	47.1 ±2.9	55.8* ±2.1	39.0 ±2.6	32.6* ±1.6	47.6 ±3.0	30.7* ±1.9	19.1* ±1.7	13.9* ±1.4	43.3 ±2.5	27.8* ±4.5	12.3* ±1.0	0.0
	20 kR Irradiated	28.7 ±2.4	48.6** ±2.2	44.1** ±2.5	49.5** ±2.6	38.4 ±2.7	36.5** ±1.9	38.0** ±2.2	32.3 ±1.7	25.7 ±2.1	43.4** ±2.7	20.1** ±0.6	7.7** ±1.4	0.0

± S.E.
* Significantly different from control
** Significantly different from 20 kR
Significance tested by student's 't' test (p=0.05)

Fig. 16: Modulation of radiation effects on pollen germination in Solanums.

Fig.16

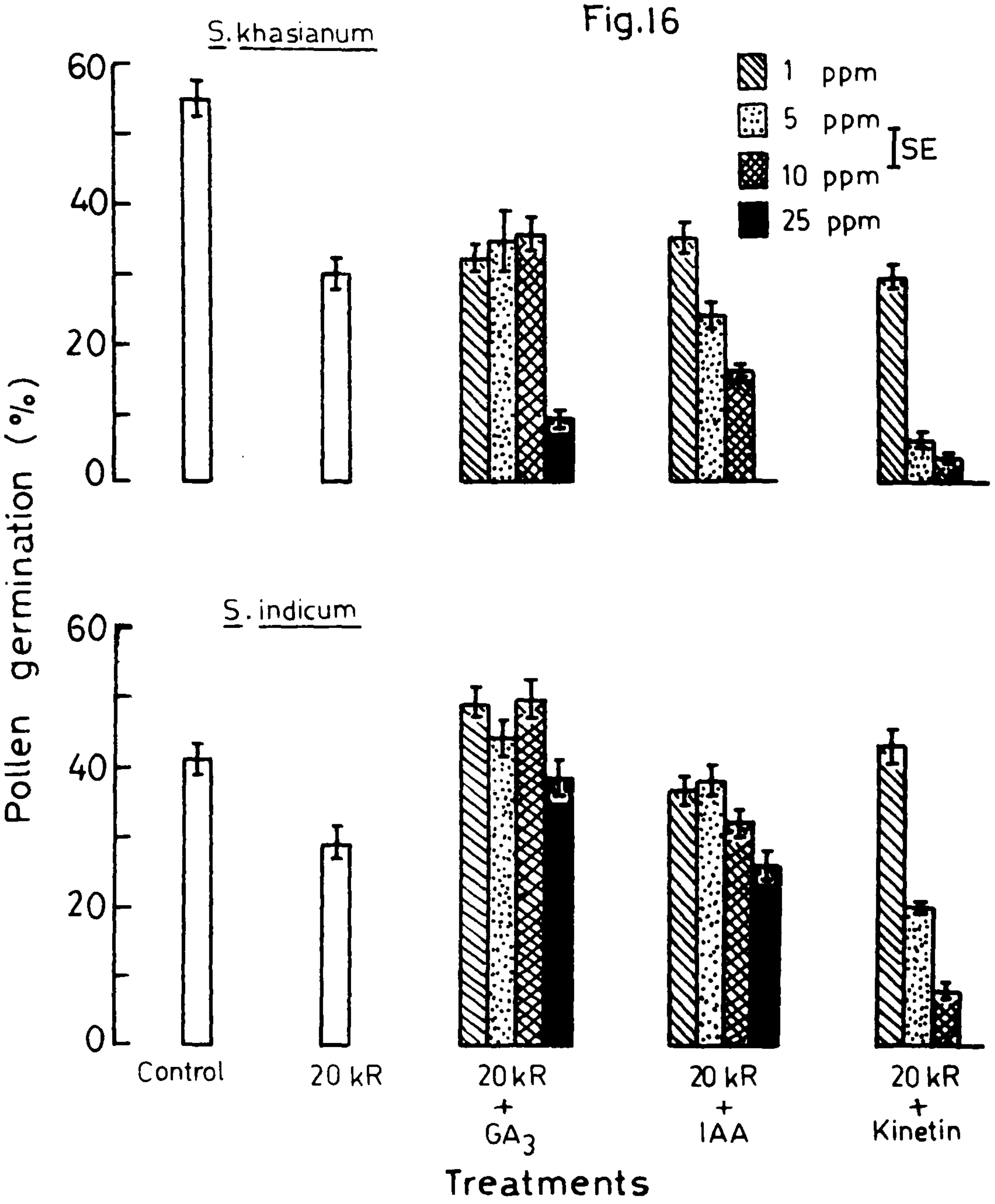

S. khasianum
S. indicum
Pollen germination (%)
60
40
20
0
▧ 1 ppm
▨ 5 ppm
▩ 10 ppm
■ 25 ppm
SE
Control
20 kR
20 kR + GA$_3$
20 kR + IAA
20 kR + Kinetin
Treatments

Plate 7 : Modulation of radiation responses of pollen germination by growth hormones in *S. khasianum* x 120

a : Control
b : 20 kR
c : 20 kR + 25 ppm GA_3
d : 20 kR + 10 ppm IAA
e : 20 kR + 25 ppm IAA
f : 20 kR + 5 ppm kinetin
g : 20 kR + 10 ppm kinetin

PLATE 7

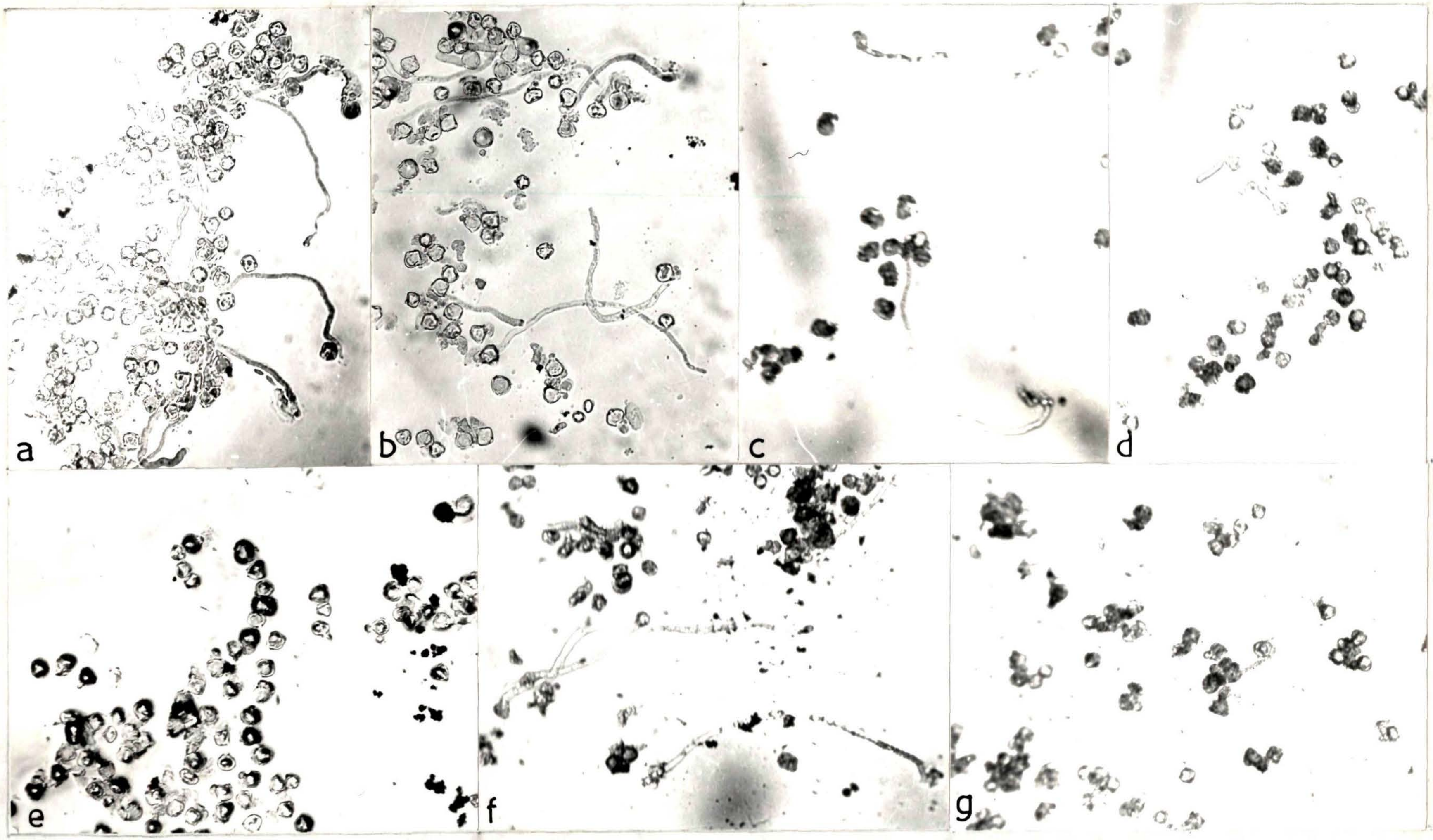


Plate 8 : Modulation of radiation responses of pollen germination by growth hormones in S. *indicum* x 120

a : Control

b : 20 kR

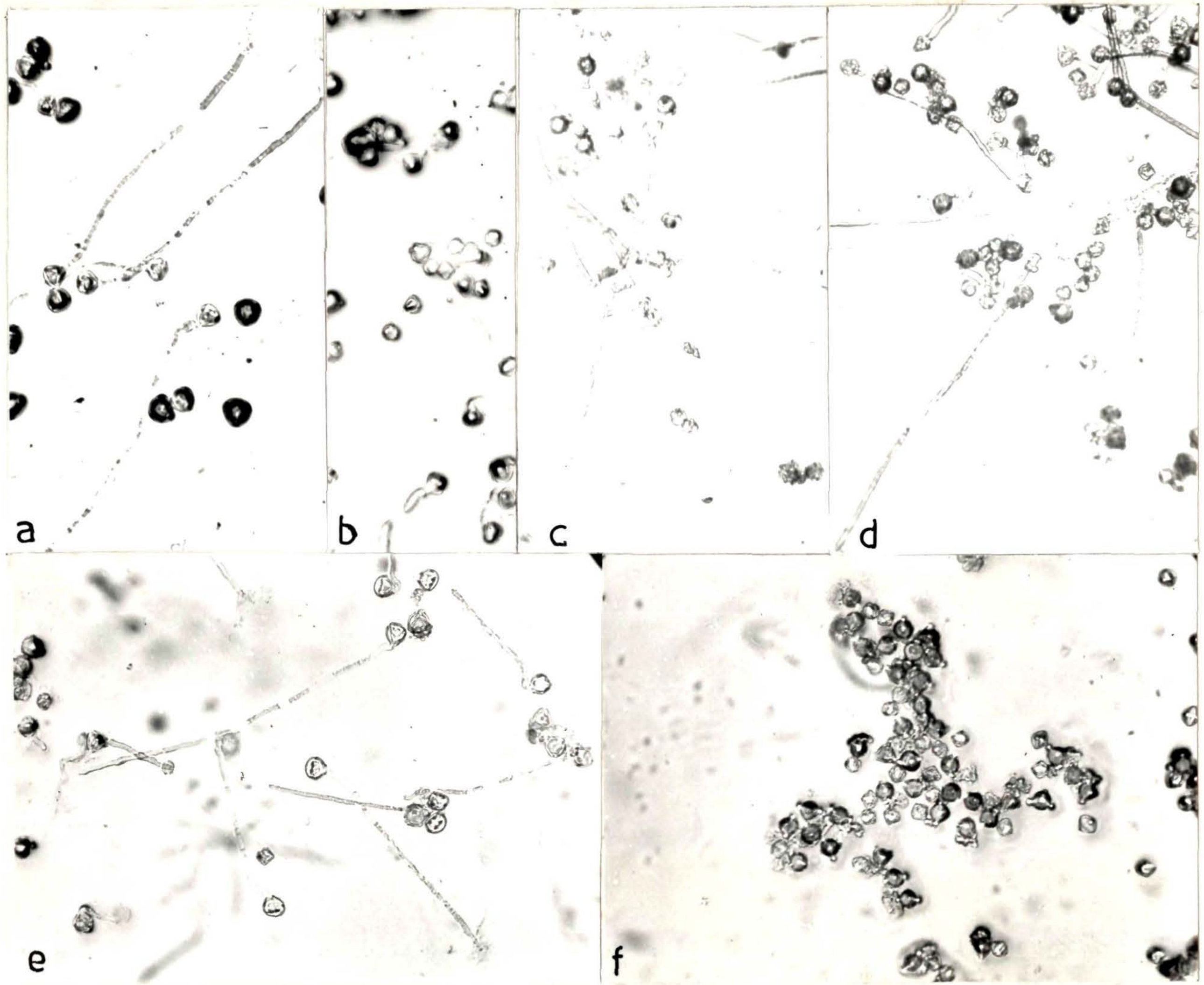
c : 20 kR + 1 ppm GA_3

d : 20 kR + 5 ppm IAA

e : 20 kR + 1 ppm kinetin

f : 20 kR + 25 ppm kinetin

PLATE 8



percentage of 41.1 (Table 25). When these pollen grains were germinated in various growth hormones (viz., GA_3, IAA and kinetin) supplemented media it was observed that though GA_3 concentrations of 1 and 5 ppm improved germination percentage the differences became significant only at 5 ppm (Table 25, Fig. 15). Ten and twentyfive ppm of GA_3 inhibited pollen germination which was significant at 25 ppm (Table 25). On the other hand, when pollen were germinated in IAA supplemented medium except 1 ppm of IAA, all the other concentrations (5, 10 and 25 ppm) significantly decreased pollen germination (Table 25, Fig. 15). A similar response was observed when various concentrations (1, 5, 10 and 25 ppm) of kinetin were used in the medium (Table 25, Fig. 15)

Only 28.7% of the pollen grains obtained from 20 kR plants had the ability to germinate compared to 41.1% germination in control pollen (Table 25, Fig. 16 and Plate 8 a,b). When the pollen grains from 20 kR plants were germinated in various growth hormones (GA_3, IAA and kinetin) supplemented media, it was observed that 1, 5 and 10 ppm of GA_3 markedly improved the germination percentage of irradiated pollen (Table 25, Fig. 16 and Plate 8 c). Twentyfive ppm of GA_3 did not alter significantly pollen germination in irradiated pollen (Table 25, Fig. 16). But when IAA was used even though 1, 5 and 10 ppm concentrations increased the germinability of irradiated pollen, the differences were significant only at the concentrations of 1 and 5 ppm (Table 25, Fig. 16 and Plate 8 d). Twentyfive ppm of IAA though enhanced radiation-induced inhibition, the differences were non-significant (Table 25, Fig. 16). On the other hand, in kinetin supplemented medium while 1 ppm of kinetin could reduce the radiation injury to pollen germination, the higher concentrations (5, 10 and 25 ppm) enhanced the radiation damage (Table 25, Fig. 16 and Plate 8 e,f). It is clear from the foregoing account that GA_3 concentrations of 1, 5 and 10 ppm, IAA concentrations of 1 and 5 ppm and kinetin concentrations of 1 ppm could radioprotect the process of pollen germination against radiation injury. GA_3 concentrations of 25 ppm and IAA concentrations of 10 and 25 ppm while did not have any appreciable influence on pollen germination, 5, 10 and 25 ppm of kinetin sensitized this process (Table 25).

B. POLLEN TUBE GROWTH

I. Gamma-ray effects :

S. khasianum :

The pollen tubes of control measured 237 µm at the end of 4 hr incubation period. Though 2.5 and 5 kR exposed pollen produced longer pollen tubes, the differences were insignificant. Ten to twenty kR exposures of gamma rays induced inhibition which was not significant at 10 kR (Table 23, Fig. 14).

S. indicum :

The pollen tubes of control measured 510 µm. In this species, irradiated pollen grains produced smaller pollen tubes compared to control, but the differences were significant only in 15 and 20 kR irradiated pollen (Table 24, Fig. 14).

II. Effect of growth hormones and modulation of radiation responses :

S. khasianum :

The control pollen tubes measured 388 µm at the end of 4 hr incubation. GA_3 concentrations of 1 and 5 ppm although had no effect on pollen tube elongation, the higher concentrations of GA_3 (10 and 25 ppm) significantly decreased pollen tube size (Table 26, Fig. 17). All concentrations of IAA and kinetin (1, 5, 10 and 25 ppm) reduced pollen tube elongation, drastically (Table 26, Fig. 17).

Twenty kR exposure of gamma rays reduced pollen tube elongation vis-a-vis control (Table 26, Fig. 18 and Plate 9 a,b). When the pollen grains obtained from plants given 20 kR exposure of gamma rays were grown in various hormone supplemented media, it was found that 1-10 ppm of GA_3 markedly improved pollen tube growth while 25 ppm of GA_3 reduced pollen tube elongation drastically (Table 26, Fig. 18 and Plate 9 c,d). Thus GA_3 concentrations upto 10 ppm acted as a radioprotector, but 25 ppm increased radiation injury caused to pollen tube elongation. All the IAA concentrations used radiosensitized pollen tube elongation and the degree of sensitization increased with increasing concentration (Table 26, Fig. 18 and Plate 9 e,f). Except the lowest concentration of kinetin (1 ppm) which was radioprotective, other concentrations (5, 10 and 25 ppm) radiosensitized the

Table 26: Effect of growth hormones and modulation of radiation responses of pollen tube growth (µm) in Solanums.

Species	Treatment	Control	GA$_3$				IAA				KINETIN			
			1 ppm	5 ppm	10 ppm	25 ppm	1 ppm	5 ppm	10 ppm	25 ppm	1 ppm	5 ppm	10 ppm	25 ppm
S. khasianum	Control	388 ±16.8	389 ±14.1	385 ±11.0	302* ±11.4	294* ±9.5	144* ±6.4	66* ±1.8	31* ±1.0	3*	293* ±13.3	92* ±5.5	0	0
	20 kR Irradiated	263 ±7.8	498** ±14.8	568** ±14.3	380** ±12.0	49** ±1.7	179** ±8.4	78** ±2.9	39** ±1.6	0	398** ±13.5	44** ±3.4	33** ±1.7	0
S. indicum	Control	586 ±15.3	641 ±25.0	731* ±25.3	596 ±19.3	417* ±13.0	548 ±16.2	391* ±14.4	290* ±9.4	47* ±3.0	397* ±10.5	118* ±6.0	75* ±4.2	0
	20 kR Irradiated	418 ±12.9	566** ±18.0	578** ±17.2	580** ±13.2	484 ±16.3	501** ±15.7	502** ±15.5	406 ±9.3	96** ±4.0	492** ±16.3	105** ±4.2	47** ±1.8	0

± S.E.
* Significantly different from control
** Significantly different from 20 kR
Significance tested by student's 't' test (p=0.05).

Fig. 17: Effect of growth hormones on pollen tube growth in Solanums.

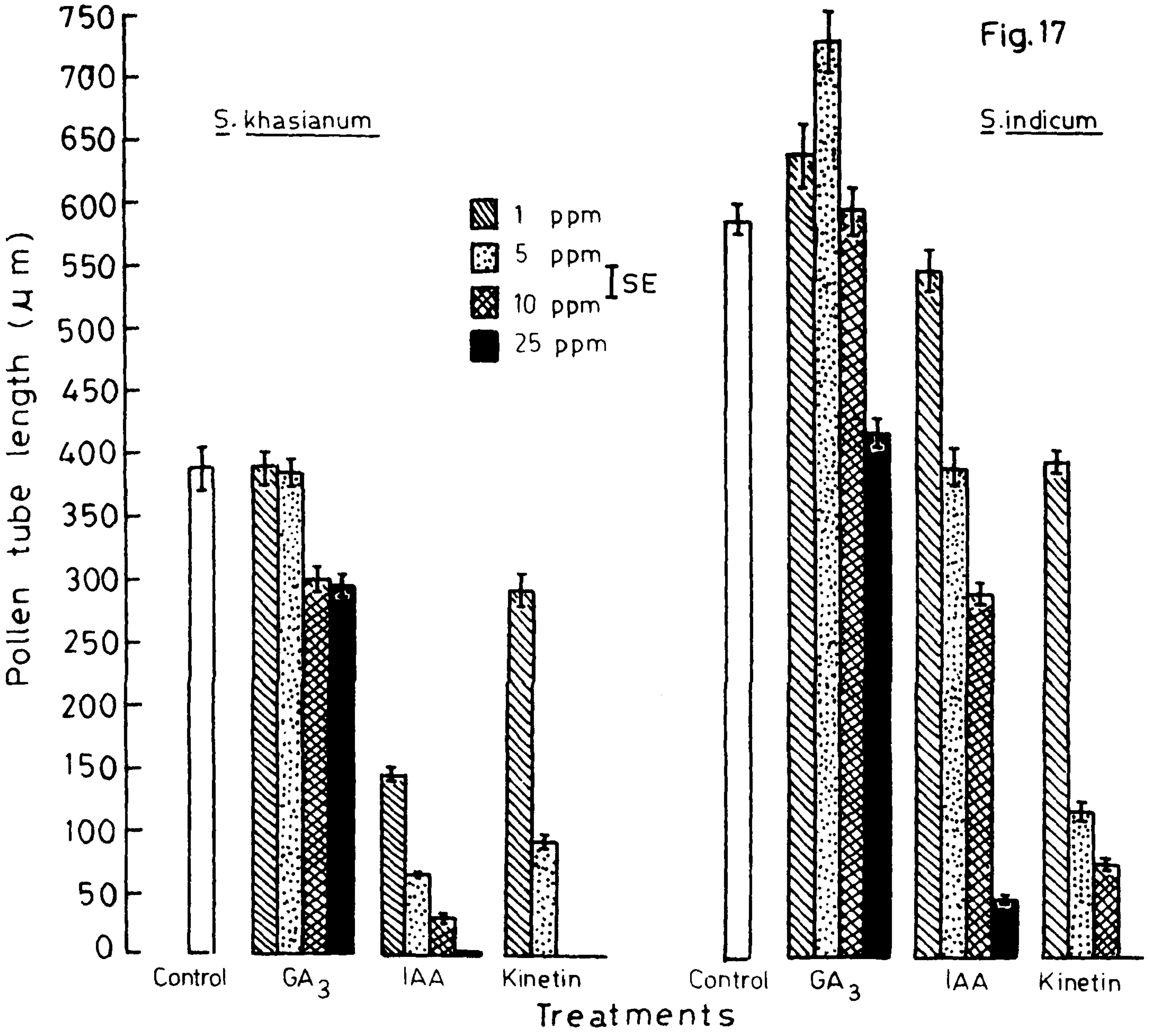
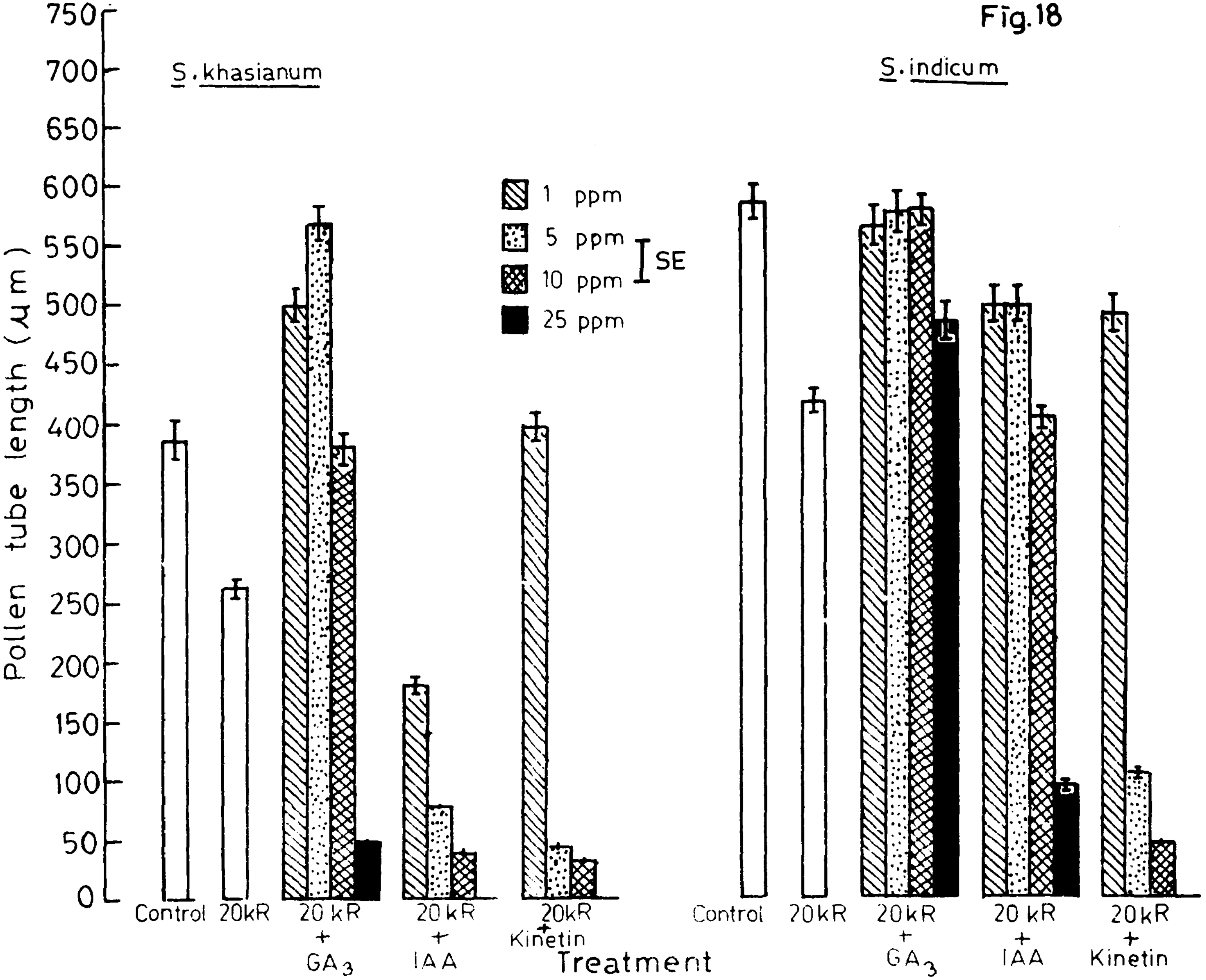


Fig. 17
Pollen tube length (μm)
750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
S. khasianum
S. indicum
▧ 1 ppm
▨ 5 ppm
▩ 10 ppm
■ 25 ppm
I SE
Control
GA$_3$
IAA
Kinetin
Control
GA$_3$
IAA
Kinetin
Treatments

Fig. 18: Modulation of radiation effects on pollen tube growth in Solanums.

Fig.18



750
700
650
600
550
500
450
400
350
300
250
200
150
100
50
0
Pollen tube length (μm)
S. khasianum
S. indicum
▨ 1 ppm
▨ 5 ppm
▨ 10 ppm
■ 25 ppm
SE
Control
20kR
20 kR + GA3
20 kR + IAA
20kR + Kinetin
Control
20kR
20kR + GA3
20 kR + IAA
20kR + Kinetin
Treatment

Plate 9 : Modulation of radiation responses of pollen tube growth by growth hormones in *S. khasianum* x 120

a : Control
b : 20 kR
c : 20 kR + 5 ppm GA_3
d : 20 kR + 25 ppm GA_3
e : 20 kR + 1 ppm IAA
f : 20 kR + 25 ppm IAA
g : 20 kR + 1 ppm kinetin
h : 20 kR + 10 ppm kinetin

PLATE 9

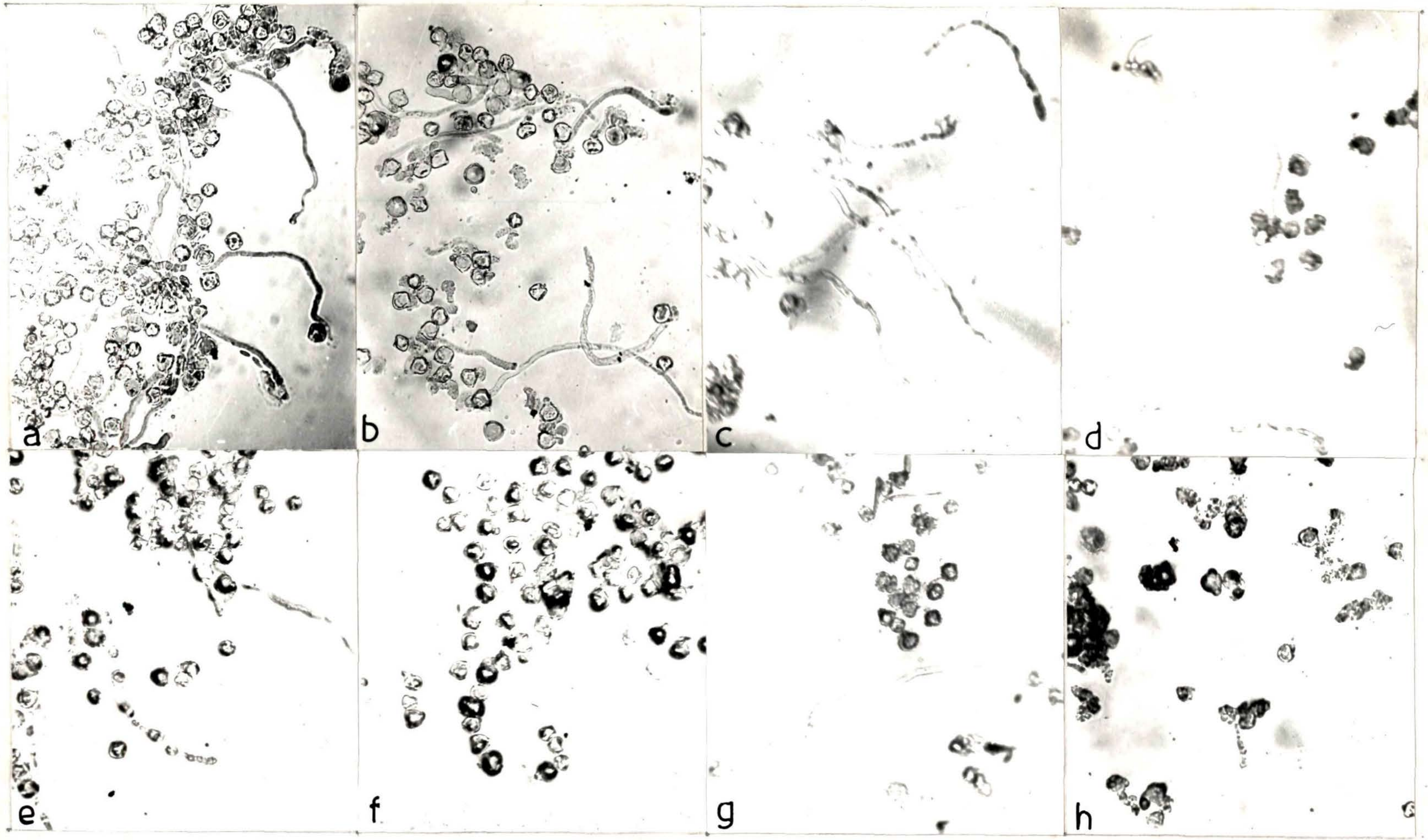
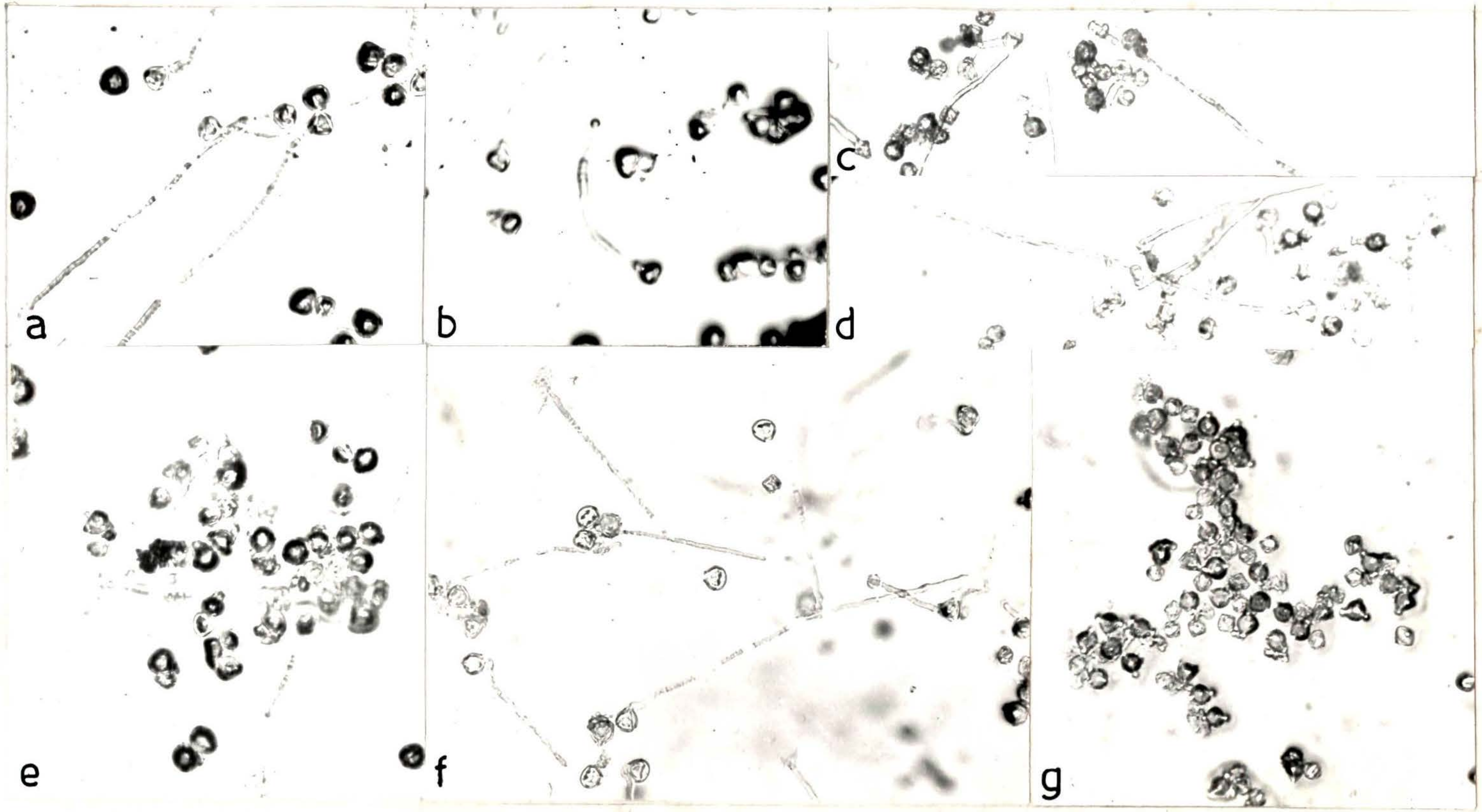


Plate 10 : Modulation of radiation responses of pollen tube growth by growth hormones in S. *indicum*

x 120

a : Control
b : 20 kR
c : 20 kR + 1 ppm GA_3
d : 20 kR + 5 ppm IAA
e : 20 kR + 25 ppm IAA
f : 20 kR + 1 ppm kinetin
g : 20 kR + 25 ppm kinetin

PLATE 10



phenomenon of pollen tube growth (Table 26, Fig. 18 and Plate 9 g, h).

S. indicum :

The control pollen tubes of S. indicum measured 586 µm. Lower concentrations of GA_3 (1, 5 and 10 ppm) though increased pollen tube elongation the stimulation was significant only at 5 ppm (Table 26, Fig. 17). Twentyfive ppm of GA_3, however, caused significant inhibition to pollen tube growth (Table 26, Fig. 17). All the concentrations of IAA reduced pollen tube elongation and the inhibition was significant except at 1 ppm (Table 26, Fig. 17). All kinetin concentrations drastically reduced pollen tube elongation (Table 26, Fig. 17).

The irradiated pollen grains produced shorter tubes compared to control (Table 26, Fig. 18 and Plate 10 a,b). The irradiated pollen grains when grown in GA_3 (1, 5 and 10 ppm) supplemented medium resulted in a marked stimulation of pollen tube elongation (Table 26, Fig. 18 and Plate 10 c). One and five ppm of IAA while induced significant recovery from radiation injury to pollen tube elongation (Table 26, Fig. 18 and Plate 10 d), 10 and 25 ppm of IAA further enhanced radiation injury which became significant at 25 ppm (Table 26, Fig. 18 and Plate 10 e). Except for 1 ppm of kinetin which reduced radiation injury to pollen tube elongation, other concentrations of kinetin enhanced the damage (Table 26, Fig. 18 and Plate 10 f,g). Thus in this species only lower concentrations of hormones were radioprotective to pollen tube elongation.

DISCUSSION

Gamma-ray effects :

Gamma-ray exposures had no appreciable influence on pollen germination in S. khasianum. In S. indicum, however, the pollen germination decreased with an increase in exposure and the LD_{50} for pollen germination was between 15 and 20 kR exposure. Swaminathan and Murthy (1959) have reported LD_{50} of 11 kR for pollen germination in Nicotiana rustica. Pfahler (1971) has observed a linear decrease in germination percentage of maize pollen following increasing exposures of gamma rays.

In both S. khasianum and S. indicum though low exposures of gamma rays had no appreciable effect on pollen tube elongation, the higher exposures (15 and 20 kR) were inhibitory. Pfahler (1971) has reported a proportionate decrease in pollen tube elongation in maize following gamma-ray exposures.

Since pollen germination and pollen tube growth is a process of cell enlargement it is considered that the primary effects of gamma rays on pollen germination and pollen tube growth may be due to :

i) Gross impairment of binding of calcium ion essential for normal tube elongation and a cumulative type of physiological action probably affecting principally the cell membrane and cell machinery involved in synthesis of cellulose, pectin and callose (Brewbaker and Emery, 1962).

ii) Impairment or destruction of cell constituents including cell membranes and enzymes following high levels of ionizing radiation (Brewbaker *et al.*, 1965).

iii) Disruption of cell membrane (Pfahler, 1971).

iv) Cessation of protein synthesis (Cresti *et al.*, 1977).

v) Inteference with the main regulatory mechanism of pollen tube growth by de- and re-masking of mainly pre-synthesized m RNA (Van der Donk *et al.*, 1978).

Thus the mechanism of gamma-ray-induced inhibition of pollen germination and pollen tube growth is complex.

Effect of growth hormones :

GA_3 treatments [redacted] significantly inhibited pollen germination and pollen tube growth in *S. khasianum*. These results find support from the findings of Dhingra and Varghese (1976) in *Lycopersicon esculentum*. In *S. indicum*, however, the response of pollen germination and pollen tube elongation to GA_3 followed an optimum curve with the peak values in 5 ppm GA_3. Promotion of pollen germination and tube elongation by GA_3 is reported in *Pisum* (Bose, 1959). GA_3-induced stimulation of pollen tube elongation may be due to promotion of cell expansion and orientation of newly synthesized cellulose microfibrils (Malik and Chhabra, 1976).

All concentrations of IAA reduced pollen germination and pollen tube elongation in *S. khasianum*. In *S. indicum* except

1 ppm of IAA, the other concentrations (5, 10 and 25 ppm) significantly reduced pollen germination and pollen tube length. These results are in agreement with those of Yadav (1980) in *Cassia tora* and *Cassia obtusifolia*.

Kinetin treatments resulted in a decrease in pollen germination and pollen tube elongation in both the species. This could be because exogenous supply of kinetin may have become toxic due to supra optimal concentration.

Modulation of radiation injury :

In *S. khasianum* GA$_3$ concentrations of 1, 5 and 10 ppm, which did not modulate radiation effect on pollen germination, significantly nullified the inhibition caused to pollen tube elongation by 20 kR exposure. However, 25 ppm GA$_3$ in the medium further enhanced radiation injury for both pollen germination and pollen tube elongation. In *S. indicum* all the GA$_3$ treatments resulted in a recovery in pollen germination and tube growth relative to 20 kR alone; the recovery was significant upto 10 ppm. Siders *et al.* (1969) have reported the destruction of biological activity of gibberellic acid by low doses of ionizing radiation. Exogenous supply of GA$_3$ in the medium resulted in a recovery of pollen germination and tube growth in case of pollen from irradiated plants. Post-irradiation modulation of radiation injury by GA$_3$ treatments is known for maize seedlings (Gaur and Notani, 1960), wheat seedlings (Haber and Luipold, 1960; El-Keredy *et al.*, 1975), pinto bean plants (Lockhart, 1961) and *Phaseolus vulgaris* (Silveira and Hell, 1977).

Indole acetic acid which inhibited pollen germination and pollen tube elongation in *S. khasianum* further enhanced the radiation injury caused to both the processes in this species. In *S. indicum*, while low concentrations of IAA (1 and 5 ppm) acted as radioprotective to both the phenomena, the higher concentration of 25 ppm significantly increased radiation injury to pollen tube elongation. The present findings thus indicate that only low concentration of auxin are able to reduce radiation-induced inhibition. These results, therefore, support the findings of Chauhan and Singh (1975), Chauhan (1976, and in press) in *Carthamus tinctorius*. Bhattacharya and Rao (1978) are of the opinion that radiation causes IAA breakdown in irradiated material by stimulating IAA oxidase activity and higher concentrations of exogenous IAA (50, 100 ppm) resulted in significant growth recovery. Post-irradiation modulation of radiation injury by exogenous supply of IAA is reported for *Crepis* (Araratyan and Azatyan, 1974), soybean (Bhattacharya, 1977), and rice (Bhattacharya and Rao, 1978).

In *S. khasianum* 1 ppm kinetin resulted in significant increase in pollen tube length but pollen germination was not affected as compared to 20 kR alone. In *S. indicum*, however, both these processes were significantly stimulated relative to 20 kR. Gamma rays are known to influence cytokinin synthesis in irradiated material (Pandey *et al.*, 1978). In both these species gamma-irradiation might have resulted in a decrease in endogenous cytokinin levels in pollen and, therefore, exogenous supply of kinetin (1 ppm) in the medium resulted in a restoration of normal growth of pollen. The higher concentration (5, 10 and 25 ppm) which further increased radiation injury in both the species may have become toxic due to supraoptimal concentration. Radioprotective effects of kinetin are reported in literature (Araratyan *et al.*, 1975; Mikhailov *et al.*, 1978; Jonard *et al.*, 1979).

CHAPTER VIII

HETEROSTYLY

INTRODUCTION

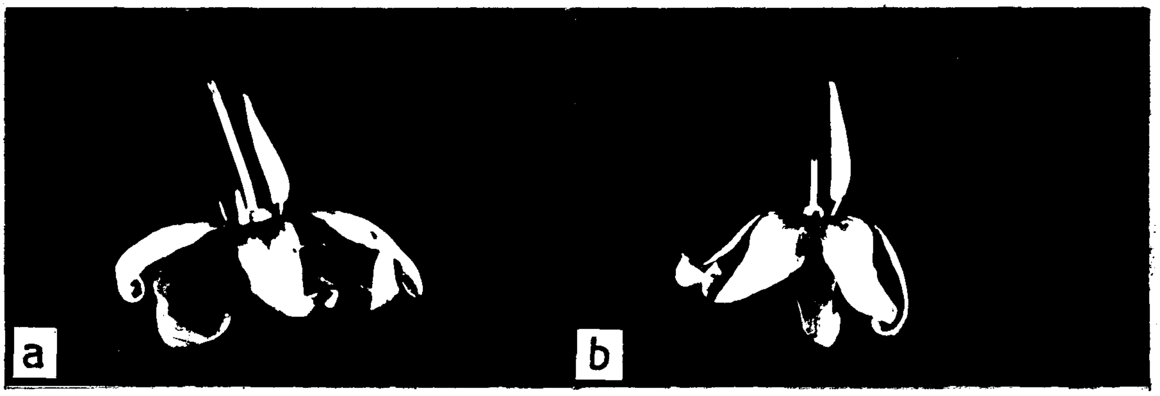
Typical heterostyly with stamens and styles in complementary position which promote outbreeding is reported for the family Solanaceae (Backer and Bakhuizen, 1965). In Solanum, however, only one stamen tier remains constant to all the different forms of style lengths. This condition is considered to be one step towards the evolution of heterostyly (Reddy and Bahadur, 1977). Opinions differ as to whether the stylar heteromorphism is determined by physiological, environmental or genetic factor independently or by a combined effect of these factors. Pal and Singh (1943) have suggested the effect of weather and season to be the main factors for the stylar variation and have reported 3 types of flowers in egg plant: long styled, pseudo-short styled and short styled. Wakhloo (1975a) in S. sisymbrifolium has shown a correlation between the short styled (female sterile) and long styled (female fertile) flowers with low and high potassium content, respectively. Murthy and Abraham (1975a) reported heterostyly in S. khasianum (Plate 11 a,b) and suggested that age of the plant has a bearing on the expression of heterostyly in this species. Hossain (1973) opined that the occurrence of stylar variation in the different flowers of the same individual clearly suggests a physiological effect rather than a genetical one. He further suggested that the constancy of the phenomenon in the species may have some kind of genetic basis, for there are some species in the genus which do not show any variation in style length eg. Solanum

Plate 11 : Heterostyly in S. khasianum

a : Long style

b : Short style

PLATE 11



verbascifolium, *S. seaforthianum* and *S. indicum*. According to Martin (1972) the reduction in style size and sterility in *S. mammosum* could be due to lack of sufficient developmental hormones. Wakhloo (1975b) has shown that the production of female sterile flowers in *S. sisymbrifolium* plants low in potassium content is enhanced by GA_3 treatment, however, kinetin application enhances development of female fertile (long styled) flowers. Reddy and Bahadur (1977) consider that the cause of female sterility of short styled flowers in *S. surattense* is probably hormonal and sporophytically determined. Heslop-Harrison (1957) while discussing the possible mechanism of sex expression in flowering plants has postulated that either a single or two different auxins control the growth of pistil and stamen. In case of a single auxin involvement the concentration of auxin responsible for the maximum pistil growth is higher than the concentration required for maximum stamen growth so that the auxin level at the differentiating apex determines the sex balance of the flowers produced. This hypothesis explains the intermittent sterility in *Cleome spinosa* where hermaphrodite, male and female flowers occur on the same inflorescence. In this species the differences in the flower sex are attributed to the oscillation of the auxin level between the stamen and pistil optima. The hermaphrodite flowers, it is conjectured, appear when the level of auxin is somewhere in between the two optima. The abortion of stamens in some pistillate flowers of this species is regarded to be due to inhibitory effect of a high auxin level which promotes pistil growth. On the other hand, where two separate auxins regulate the stamen and pistil growth the level of auxin responsible for maximum stamen growth remains constant rendering the stamen length, pollen fertility and pollen size more or less the same from base to tip of the inflorescence and the level of auxin responsible for pistil growth fluctuates randomly at different places in the inflorescence or among different inflorescences in the same plant, thus resulting in heteromorphic development of styles. Such a condition is speculated for S. macranthum (Baksh and Iqbal, 1978). Thus possibly more than one mechanism influence the stylar development in Solanums.

Chauhan and Ravindran (1979) studied the relationship between heterostyly and berry production in S. khasianum and reported that berry production could be enhanced by increasing the incidence of long styled flowers. Since information on the mechanism of heterostyly in S. khasianum is scanty and as berry is the source of alkaloid an attempt was made to elaborate the mechanism involved in the expression of heterostyly in this species. The study was confined to S. khasianum only as S. indicum does not show any stylar variation (Hossain, 1973) and the occurrence of short styled flowers is very rare.

The present study deals with the following aspects of heterostyly as evident by the development of long styles:

I. Effect of age and season on the occurrence of long styled flowers.

II. Hormonal regulation of heterostyly.

III. Effect of gamma-ray exposures on heterostyly and modulation of radiation responses.

MATERIALS AND METHODS

I. Effect of age and season on the occurrence of long styled flowers

Solanum khasianum plants raised from control seeds and maintained at the experimental beds of the Botanical Garden of North-Eastern Hill University, Shillong, were used in the present experiment. Data were recorded on 10 plants. The plants began to flower from July onwards. Ten clusters per plant were tagged at the beginning of every month and the subsequent observations for the incidence of long and short styled flowers were recorded in these tagged clusters. These observations were made between July and October as in November, with the onset of winter, very few flowers were produced. A monthly record of the maximum and minimum temperature prevailing in Shillong during these months (July - October) was also maintained.

II. Hormonal regulation of heterostyly:

In 1979 a preliminary experiment was conducted with three plant hormones gibberellic acid (GA_3) (Phylaxia, Budapest), Indole acetic acid (IAA) (E. Merck, India) and 6-furfurylamino-purine(Kinetin) (Loba-Chemie, Austria). Seventyfive days old seedlings of control S. khasianum were transplanted in pots filled with 1:1 mixture of soil and farmyard manure. The first buds appeared about 90 days after sowing. Hormonal sprays were carried out from this stage onwards and were repeated at fortnightly intervals. The aforementioned hormones, each in a strength of 100 ppm, were used as foliar spray. The stock solutions of plant growth hormones were prepared as per details given below :

For GA_3 and IAA solutions 100 mg each of GA_3 and IAA were first dissolved in a few drops of ethanol and the volume made upto 1000 ml with double glass distilled water in a volumetric flask to get a 100 ppm stock solution. For kinetin solution 100 mg of kinetin was first dissolved in a few drops of 1N HCl and the volume was made upto 1000 ml with double glass distilled water in a volumetric flask to get a 100 ppm stock solution.

Care was taken to segregate the treatments and the entire plant was thoroughly doused with the hormonal spray until to the point of run off. Control plants were sprayed with double glass distilled water. Five plants were maintained per treatment and the treatments were randomized. The percentage occurrence of long styled flowers were calculated at the end of each spraying. Since the flowers began to anthesize only after the second spray, the data could be recorded only from second spray onwards.

Based on the information gathered from preliminary experiment, a more elaborate experiment was set in 1980 using three concentrations (25, 50 and 100 ppm) each of IAA and kinetin. Besides, 100 ppm of an antiauxin maleic hydrazide (Leopold and Klein, 1952) and 50 ppm of Rifampicin* were also used to elucidate if IAA or kinetin or both have any role in heterostyly. The stock solutions of maleic hydrazide (MH) (Indian Drugs and Pharmaceuticals Limited, Hyderabad) and Rifampicin (Biochem Pharmaceutical Industries, Bombay) were prepared as per details given below:

For maleic hydrazide solution 100 mg of MH was first dissolved in a few drops of ethanol and the volume made upto 1000 ml with double glass distilled water in a volumetric flask to get a 100 ppm stock solution. For Rifampicin solution 50 mg was dissolved in [redacted] double glass distilled water and the volume made upto 1000 ml in a volumetric flask to get a 50 ppm stock solution. The mode of treatment and data recording was as in 1979. Besides the average number of berries produced per plant was calculated for control and the different treatments at maturity.

Since in 1980 uniform flowering in different treatments occurred only at the end of third spraying the data was recorded from third spray onwards.

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* Cytokinins are known to act in plants by stimulating RNA and protein synthesis. Further, inhibitors of transcription and translation counteract the effect of cytokinins (Hess, 1975). Therefore, Rifampicin, which inhibits action of RNA polymerase thus stopping m-RNA and protein synthesis (Michel- Briand, 1978) was used to confirm the involvement of kinetin in long style production.

III. Effect of gamma-ray exposures on heterostyly and modulation of radiation responses:

Plants of S. khasianum raised from gamma-irradiated seeds and maintained at the experimental beds of the Botanical Garden of North-Eastern Hill University, Shillong, were used for the present experiment. Details of seed irradiation and field planting are given in Chapters III and IV. To study the effect of different exposures (2.5, 5, 10, 15, and 20 kR) of gamma rays on heterostyly 350-400 flowers from 10 plants per treatment were analysed for the incidence of long and short styled flowers. The average number of berries produced per plant was also recorded for control and irradiated plants.

Phytohormones are known to modulate radiation responses in irradiated material (Gaur and Notani, 1960; Araratyan and Azatyan, 1974; Bhattacharya, 1977; Bhattacharya and Rao, 1978). In the present study an attempt was made to modulate the radiation responses of heterostyly in 20 kR irradiated plants, since this exposure reduces long style development. A preliminary experiment was conducted in 1979 with 100 ppm each of three plant hormones (GA_3, IAA and kinetin) to find out if any of the hormone helps in the recovery of radiation damage to long style flower production in S. khasianum. A more elaborate experiment using three concentrations (25, 50 and 100 ppm) of IAA and kinetin was, however, set in 1980 season. GA_3 was omitted because it did not influence radiation responses during 1979 experiment. The methodology of planting, treatment and observation for both 1979 and 1980 experiments were similar to the respective experiments dealing with the hormonal regulation of heterostyly and described above. In this experiment, however, both control (unirradiated) and 20 kR irradiated plants served as control.

RESULTS

I. Effect of age and season on the occurrence of long styled flowers:

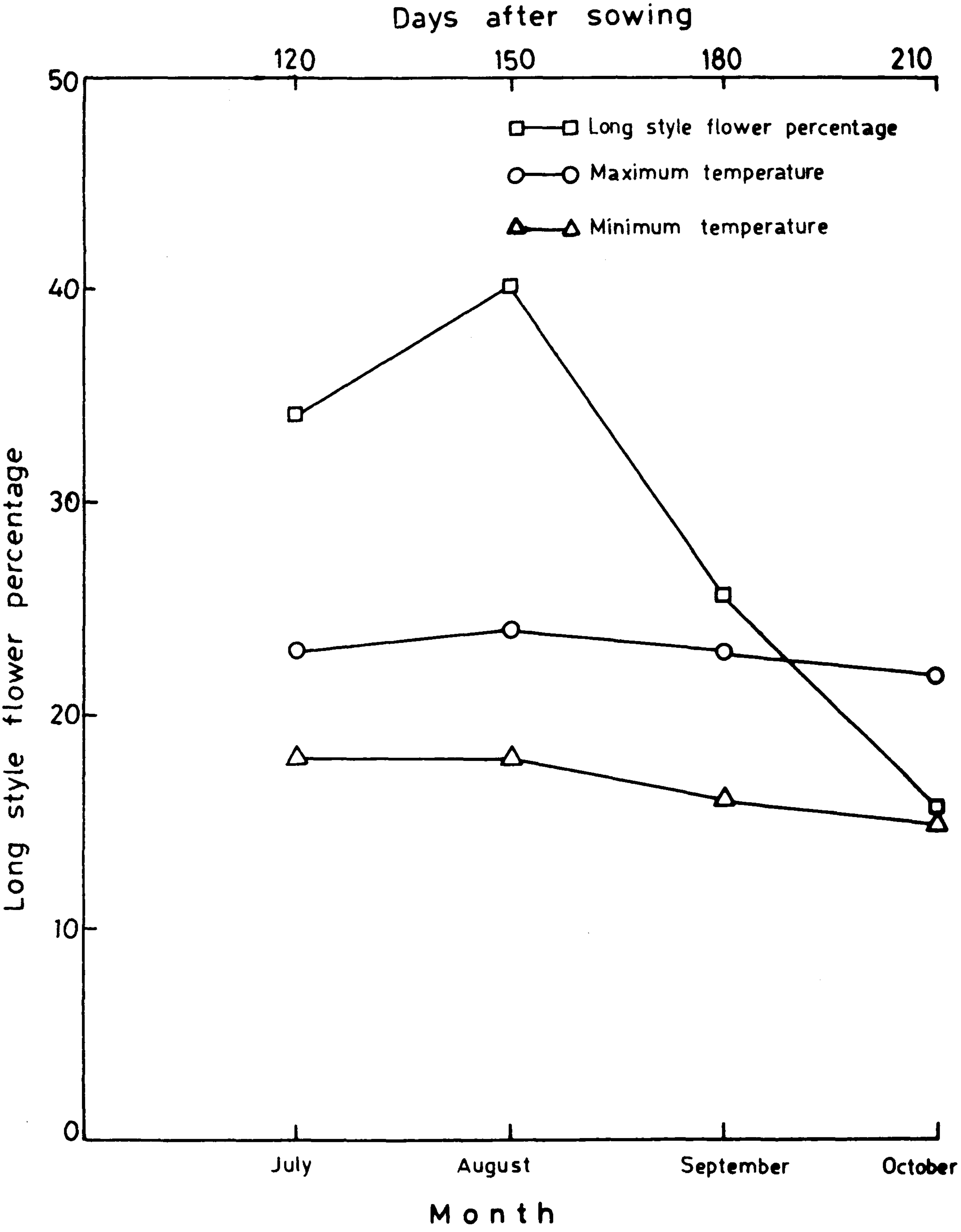
Figure 19 shows the percent occurrence of long styled flowers in different months in *S. khasianum*. The percent occurrence of long styled flowers in July was 34.1. There was a slight increase in the production of long styled flowers in August, subsequent to which it decreased considerably. Thus the incidence of long styled flowers decreased, with an increase in age of the plant, during later phase of flowering. The relationship between incidence of long styles and temperature is shown in figure 19. It is evident that the incidence of long styles is also influenced by changes in the maximum temperature. The higher temperature favoured the incidence of long styles while lower temperature favoured the incidence of short styles.

II. Hormonal regulation of heterostyly:

In the preliminary experiment of 1979, in control at the end of second spray 43 percent flowers were long styled and the percent occurrence of long styled flowers decreased with the increasing age of the plant. At the end of fifth spray the

Fig. 19: Effect of age and temperature on the occurrence of long styled flowers in S. khasianum.

Fig.19


Days after sowing
120
150
180
210
50
40
30
20
10
0
Long style flower percentage
Long style flower percentage
Maximum temperature
Minimum temperature
July
August
September
October
Month

incidence of long styled flowers in control was only 25.7 percent (Table 27). A similar trend was evident in hormone-sprayed plants also (Table 27). However, compared to control, GA_3 treatment reduced the occurrence of long styled flowers. Further, both IAA and kinetin treatments did not alter significantly the incidence of long styled flowers (Table 27), suggesting that the concentration used may be supraphysiological.

In 1980, therefore, omitting GA_3 an elaborate experiment was set using three concentrations (25, 50, and 100 ppm) of IAA and kinetin and 100 ppm MH and 50 ppm Rifampicin to find out if IAA/kinetin has any effect on the production of long styled flowers.

In control 68.6 percent of the flowers were long styled at the end of third spraying. The percentage occurrence of long styled flowers decreased with an increase in the age of the plant and at the end of fifth spray 53.4 percent of the flowers were long styled (Table 28).

Twentyfive ppm of IAA sprays resulted in increase in the production of long styled flowers (Table 28, Fig. 20). The increased incidence which differed significantly from control at the end of third and fourth sprays became non-significant at the end of fifth spray (Table 28). Fifty and hundred ppm of IAA did not alter the formation of long styled flowers, compared to control (Table 28, Fig. 20). Hundred ppm of MH-sprayed plants also did not differ significantly from control (Table 28, Fig. 20). Though the berry number in 25 ppm IAA treated plants was

Table 27: Effect of growth hormones on heterostyly in <u>Solanum khasianum</u> during 1979.

Treatment	LONG STYLE PERCENTAGES			
	2nd Spray	3rd Spray	4th Spray	5th Spray
CONTROL	43.0 ± 3.0	34.0 ± 3.5	28.5 ± 4.6	25.7 ± 4.4
GA_3	24.2 ±14.0	31.9 ± 3.4	17.9 ± 1.1	14.8* ± 1.3
IAA	36.1 ± 5.0	35.7 ± 1.6	24.2 ± 1.1	21.3 ± 1.0
KINETIN	39.3 ± 7.0	38.4 ± 3.2	29.0 ± 2.1	23.8 ± 1.8

± S.E.

* Significantly different from control

Significance tested by student's 't' test (p=0.05)

Table 28: Effects of growth hormones, MH and Rifampicin on heterostyly in *Solanum khasianum* during 1980.

Treatment	LONG STYLE PERCENTAGES 3rd Spray	 4th Spray	 5th Spray
CONTROL	68.6 ± 4.3	60.7 ± 3.6	53.4 ± 2.6
25 ppm IAA	87.2* ± 3.9	76.3* ± 4.0	63.0 ± 3.5
50 ppm IAA	63.9 ± 4.3	59.2 ± 4.9	55.1 ± 4.4
100 ppm IAA	53.6 ± 5.3	51.0 ± 3.0	47.8 ± 2.7
100 ppm MH	78.7 ±11.9	73.3 ± 9.0	69.4 ± 6.9
25 ppm KINETIN	77.6 ± 7.6	65.3 ± 7.3	55.8 ± 5.6
50 ppm KINETIN	87.4* ± 2.3	75.3* ± 2.6	64.7* ± 2.7
100 ppm KINETIN	69.0 ± 9.3	62.3 ± 7.3	54.2 ±11.0
50 ppm RIFAMPICIN	52.7$^{*-\circ}$ ± 2.3	47.6$^{*-\circ}$ ± 2.4	44.9$^{*\circ}$ ± 2.4

± S.E.

* Significantly different from control

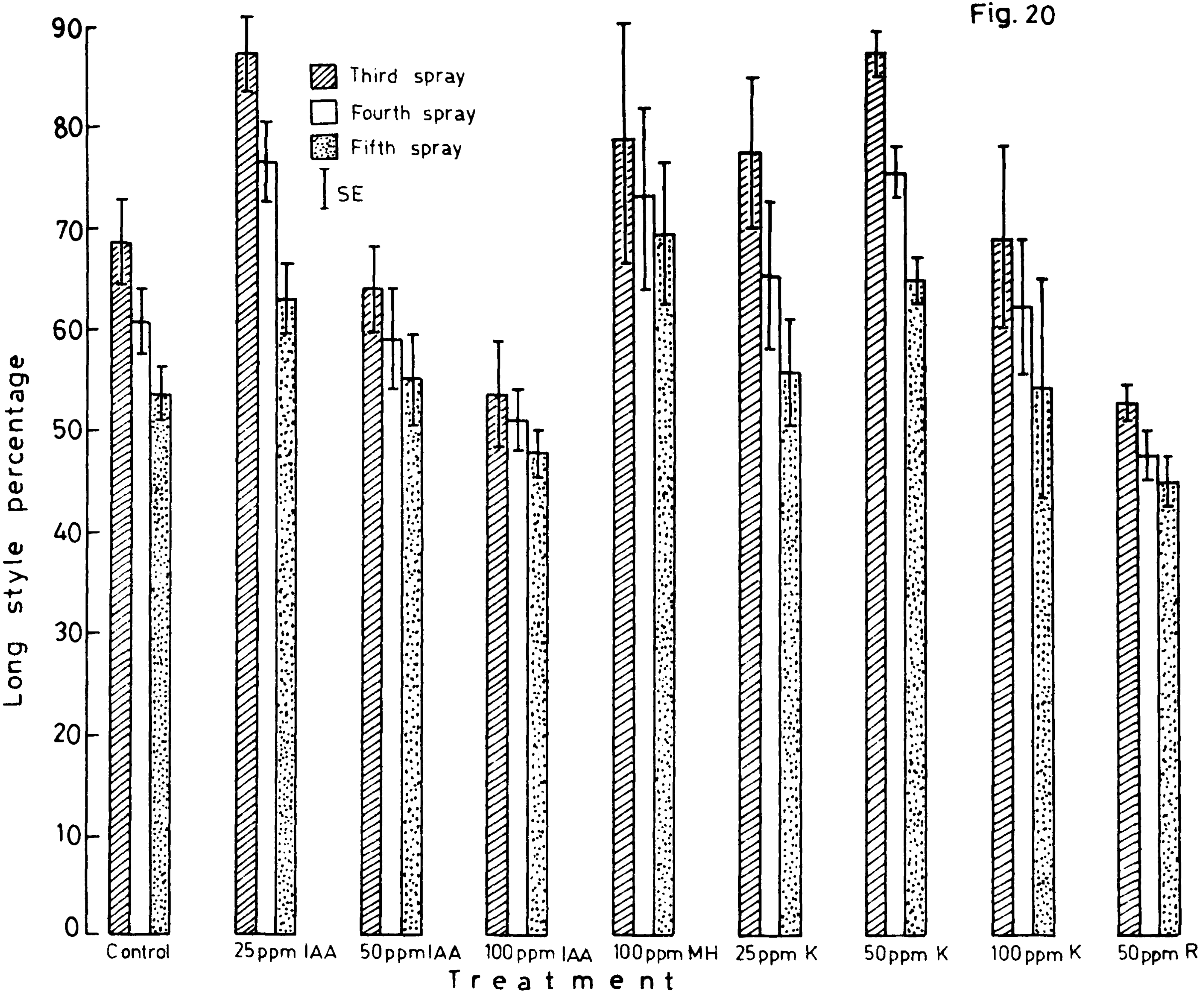
\- Significantly different from 25 ppm kinetin

° Significantly different from 50 ppm kinetin

Significance tested by student's 't' test ($p=0.05$)

Fig. 20: Effect of growth hormones, MH and Rifampicin on long style development in S. *khasianum*.

Fig. 20


Third spray
Fourth spray
Fifth spray
SE
Long style percentage
0
10
20
30
40
50
60
70
80
90
Control
25 ppm IAA
50 ppm IAA
100 ppm IAA
100 ppm MH
25 ppm K
50 ppm K
100 ppm K
50 ppm R
Treatment

more than control, 50 and 100 ppm of IAA reduced the berry number (Table 29, Fig. 21). All these differences did not differ significantly from control plants (Table 29, Plate 12a-d). In the MH treated plants the berry number was same as in control (Table 29, Fig. 21 and Plate 12 e).

All the kinetin treatments though enhanced long style production the differences with control were significant only in 50 ppm kinetin (Table 28, Fig. 21). The incidence of long styled flowers in 50 ppm Rifampicin treated plants was much lower than both control and kinetin sprayed plants (Table 28, Fig. 21). Further, while kinetin sprayed plants either bore more berries or same as control, in Rifampicin treated plants the berry number was less than control (Table 29, Fig. 21 and Plate 12 f-i). Moreover, the differences vis-a-vis control were significant only in 50 ppm kinetin sprays and 50 ppm Rifampicin sprayed plants (Table 29).

IIIa. Gamma-ray effect on heterostyly:

In this experiment the percent occurrence of long styled flowers in control was 43.3. Gamma-ray exposures promoted long style development in irradiated plants. It is clear from table 30 that the long style production increased with increasing exposure upto a threshold level subsequent to which a declining trend set in. The maximum long style production was recorded in 5 kR exposed plants and the value differed significantly from control (Table 30). The differences in the other treatments

Table 29: Effect of growth hormones, MH and Rifampicin on berry production in *Solanum khasianum* during 1980.

Treatment	Berry number/plant
CONTROL	8 ± 0.57
25 ppm IAA	10 ± 0.57
50 ppm IAA	7 ± 0.77
100 ppm IAA	6 ± 0.77
100 ppm MH	8 ± 0.63
25 ppm KINETIN	9 ± 0.57
50 ppm KINETIN	11* ± 0.72
100 ppm KINETIN	8 ± 0.57
50 ppm RIFAMPICIN	5* ± 0.75

± S.E.

* Significantly different from control

Significance tested by student's 't' test ($p=0.05$)

Fig. 21: Effect of growth hormones, MH and Rifampicin on berry production in S. *khasianum*.

Fig. 21

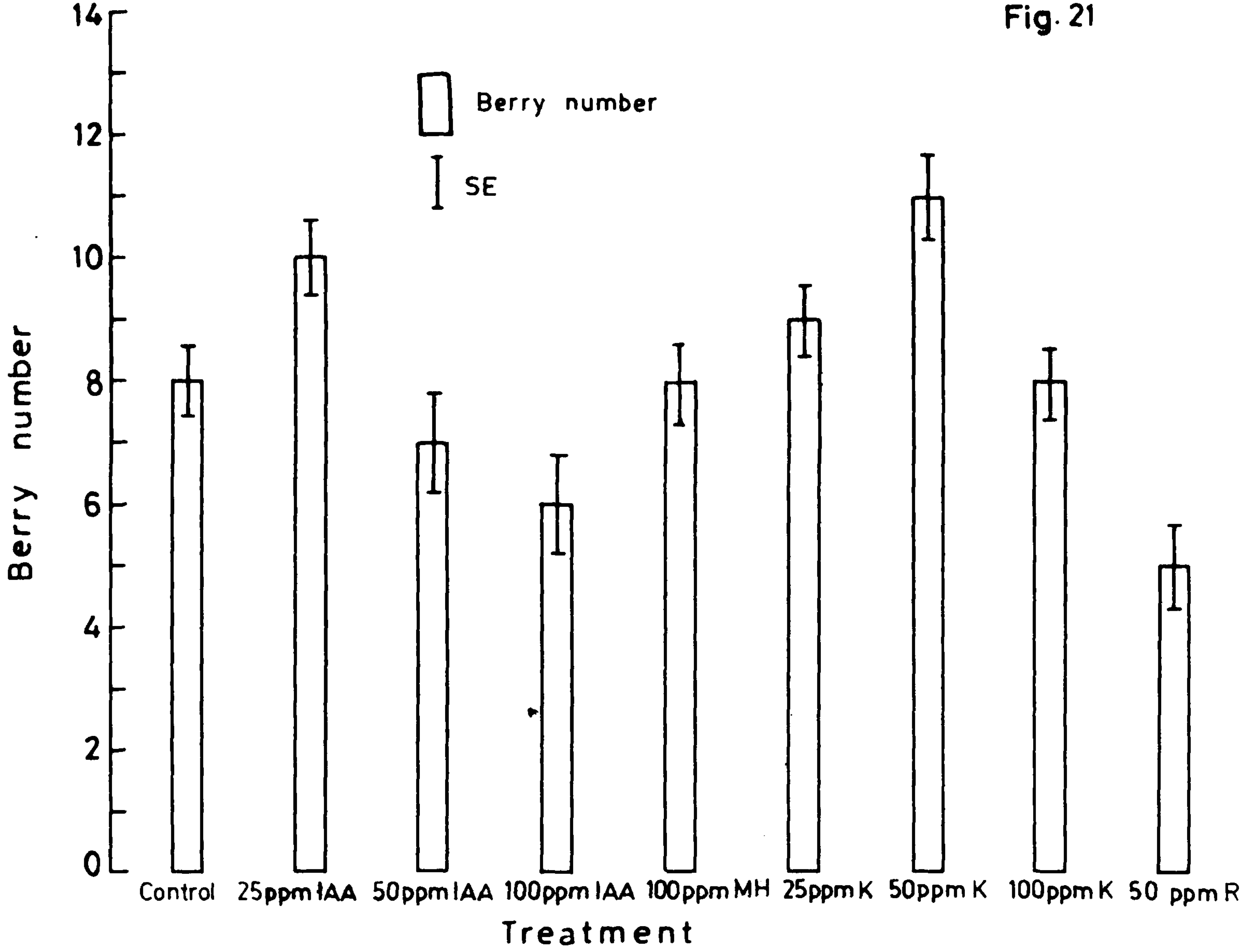

Berry number
SE
Berry number
0
2
4
6
8
10
12
14
Control
25ppm IAA
50ppm IAA
100ppm IAA
100ppm MH
25ppm K
50ppm K
100ppm K
50 ppm R
Treatment

Plate 12 : Effect of growth hormones, MH and Rifampicin on berry production in *S. khasianum* during 1980.

PLATE 12

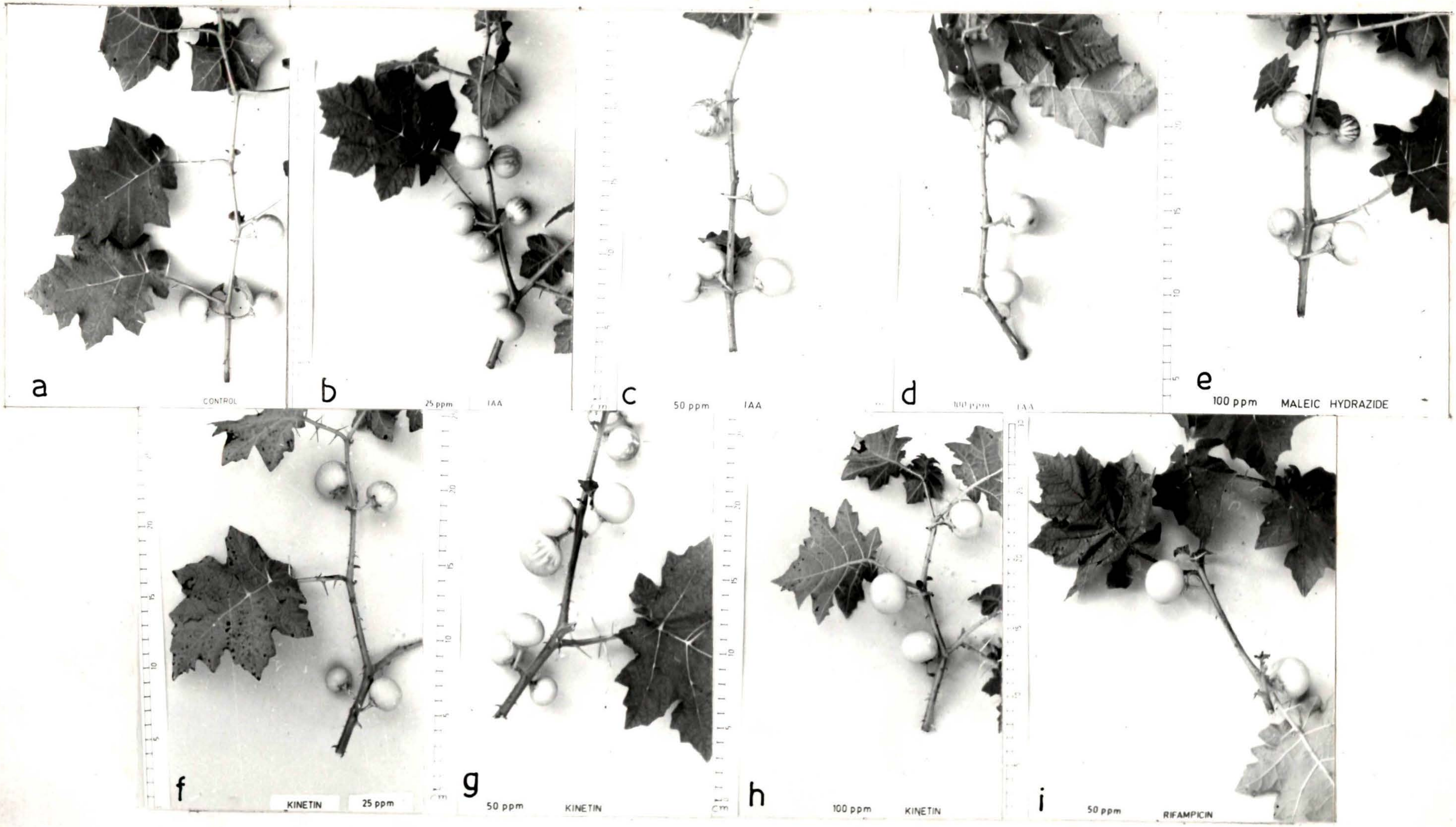

a
CONTROL
b
25 ppm
IAA
c
50 ppm
IAA
d
100 ppm
IAA
e
100 ppm
MALEIC HYDRAZIDE
f
KINETIN
25 ppm
g
50 ppm
KINETIN
h
100 ppm
KINETIN
i
50 ppm
RIFAMPICIN

Table 30: Effect of gamma-ray exposures on heterostyly and berry number per plant in <u>Solanum khasianum</u>.

Treatment	Long style frequency (%)	Berry number/plant
CONTROL	43.3 ± 5.7	62.0 ± 5.3
2.5 kR	46.6 ± 7.1	79.7 ± 8.4
5.0 kR	68.0* ± 5.1	128.4 ± 45.0
10.0 kR	57.7 ± 8.8	92.5 ± 13.6
15.0 kR	53.0 ± 7.6	70.6 ± 10.1
20.0 kR	52.7 ± 4.0	73.0 ± 15.0

± S.E.

* Significantly different from control

Significance tested by student's 't' test ($p=0.05$).

were not significant (Table 30). The increased incidence of long styled flowers was reflected in the enhanced berry production also (Table 30).

IIIb. Modulation of radiation responses:

In the preliminary experiment, compared to control, 20 kR irradiated plants produced lesser number of long styled flowers. These differences which were significant at the end of second spray, however, became non-significant in the later stages of development (Table 31).

The incidence of long styled flowers in the combinations of 20 kR + GA_3 and 20 kR + IAA did not alter vis-a-vis control or plants given 20 kR exposure (Table 31). However, the combination of 20 kR + kinetin influenced the production of long styled flowers favourably and helped in the recovery of radiation injury. This is evident from the fact that while 20 kR + kinetin differs significantly from 20 kR irradiated plants, this treatment did not differ from control (Table 31).

The results of detailed experiment conducted in 1980 for modulating radiation injury in <u>S. khasianum</u> are presented in table 32, and figure 22. The 20 kR irradiated plants produced lesser number of long styled flowers compared to control (Table 32, Fig. 22) and the berry number was also less than control (Table 33, Fig. 23 and Plate 13 a,b).

Combination treatments of 25 and 50 ppm IAA with 20 kR

Table 31: Modulation of radiation effects on heterostyly in *Solanum khasianum* by growth hormones during 1979.

Treatment	LONG STYLE PERCENTAGES			
	2nd Spray	3rd Spray	4th Spray	5th Spray
CONTROL	43.0 ± 3.0	34.0 ± 3.5	28.5 ± 4.6	25.7 ± 4.4
20.0 kR	23.0* ± 4.0	29.5 ± 4.3	23.4 ± 3.2	20.4 ± 2.2
20.0 kR+GA_3	25.0 ± 3.5	25.4 ± 2.6	17.6 ± 1.5	15.6 ± 1.3
20.0 kR+IAA	25.7 ± 1.1	27.6 ± 1.3	20.7 ± 0.7	18.9 ± 1.0
20.0 kR + KINETIN	51.8** ± 4.9	43.0** ± 2.6	36.4** ± 2.4	31.3** ± 2.0

± S.E.

* Significantly different from control

** Significantly different from 20.0 kR

Significance tested by student's 't' test (p=0.05)

Table 32: Modulation of radiation effects on heterostyly in *Solanum khasianum* by growth hormones during 1980.

Treatment	LONG STYLE PERCENTAGES		
	3rd Spray	4th Spray	5th Spray
CONTROL	68.6 ± 4.3	60.7 ± 3.6	53.4 ± 2.6
20.0 kR	49.5* ± 4.5	44.8* ± 2.3	38.1* ± 2.1
20.0 kR + 25 ppm IAA	68.3** ± 6.1	55.2 ± 4.2	48.3 ± 3.3
20.0 kR + 50 ppm IAA	71.4** ± 5.8	49.1 ± 7.5	44.9 ± 3.8
20.0 kR + 100 ppm IAA	38.9 ± 2.5	36.0 ± 2.5	32.8 ± 2.3
20.0 kR + 25 ppm KINETIN	66.7 ± 5.0	58.1 ± 4.6	49.0** ± 3.1
20.0 kR + 50 ppm KINETIN	63.1 ± 8.8	60.2 ± 6.1	51.4** ± 4.7
20.0 kR + 100 ppm KINETIN	80.3** ± 5.2	67.3** ± 3.9	59.2** ± 2.8

± S.E.

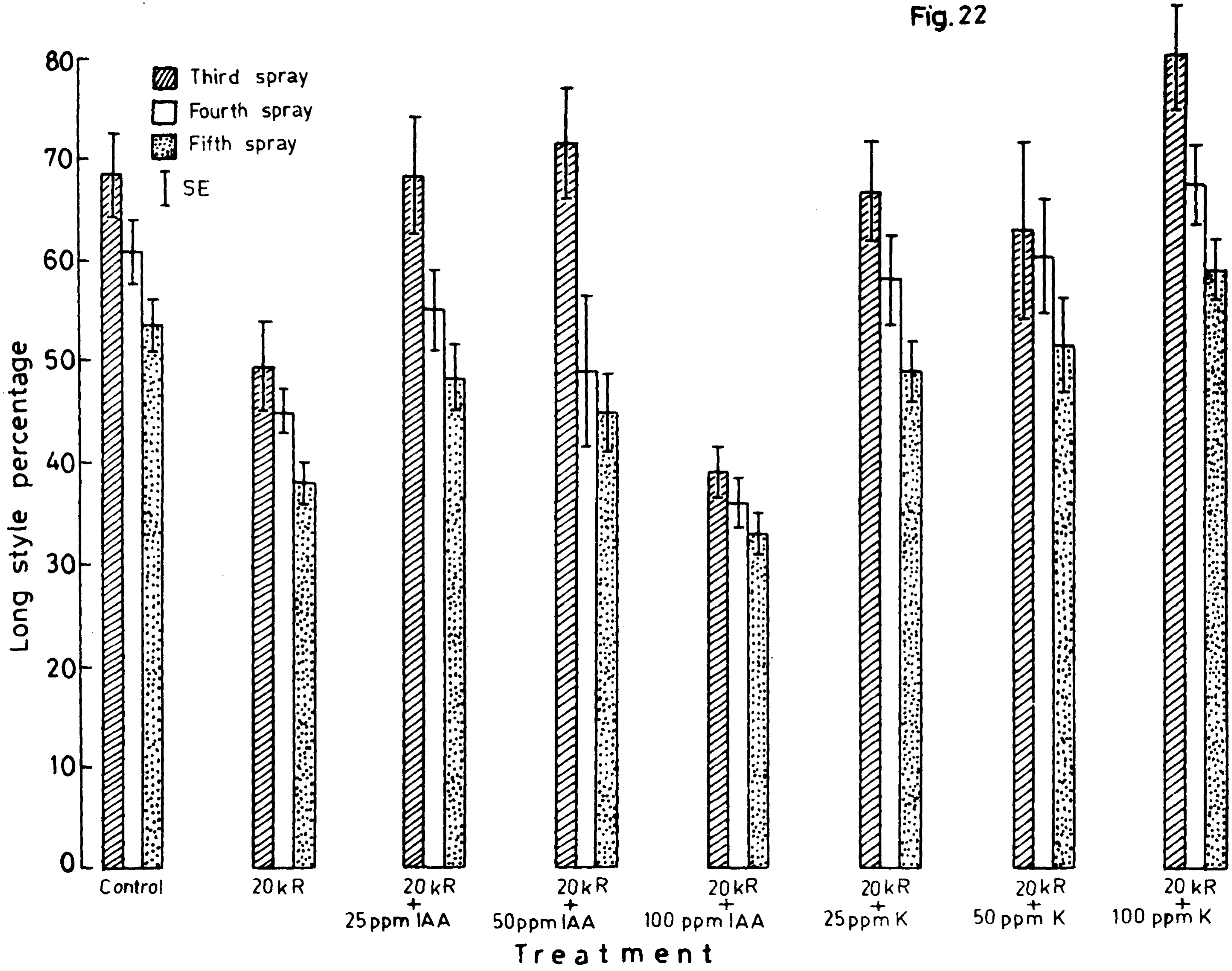
* Significantly different from control

** Significantly different from 20 kR

Significance tested by student's 't' test (p=0.05)

Fig. 22: Modulation of radiation injury by hormones in *S. khasianum*.

Fig. 22



Third spray
Fourth spray
Fifth spray
SE
Long style percentage
0
10
20
30
40
50
60
70
80
Control
20 kR
20 kR + 25 ppm IAA
20 kR + 50ppm IAA
20 kR + 100 ppm IAA
20 kR + 25 ppm K
20 kR + 50 ppm K
20 kR + 100 ppm K
Treatment

Table 33: Modulation of radiation effects on berry development in Solanum khasianum by growth hormones during 1980.

Treatment	Berry number/plant
CONTROL	8 ± 0.57
20.0 kR	5* ± 0.89
20.0 kR + 25 ppm IAA	9** ± 0.78
20.0 kR + 50 ppm IAA	7 ± 1.39
20.0 kR + 100 ppm IAA	4 ± 0.36
20.0 kR + 25 ppm KINETIN	11** ± 1.58
20.0 kR + 50 ppm KINETIN	10** ± 1.36
20.0 kR + 100 ppm KINETIN	12** ± 0.70

± S.E.

* Significantly different from control

** Significantly different from 20.0 kR

Significance tested by student's 't' test (p=0.05)

Fig. 23: Modulation of radiation effects on berry development in _S._ _khasianum_ by growth hormones.

Fig.23

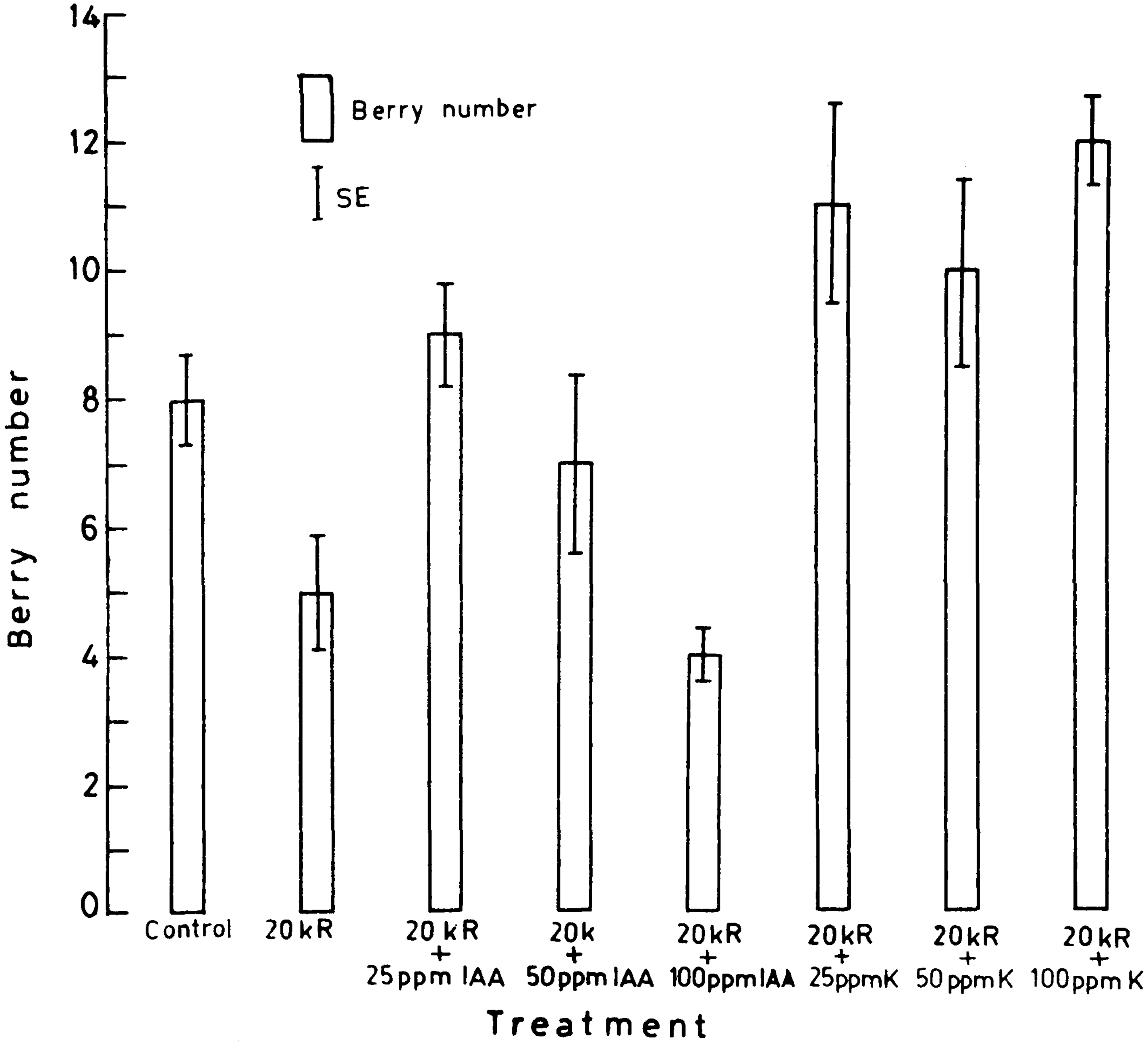
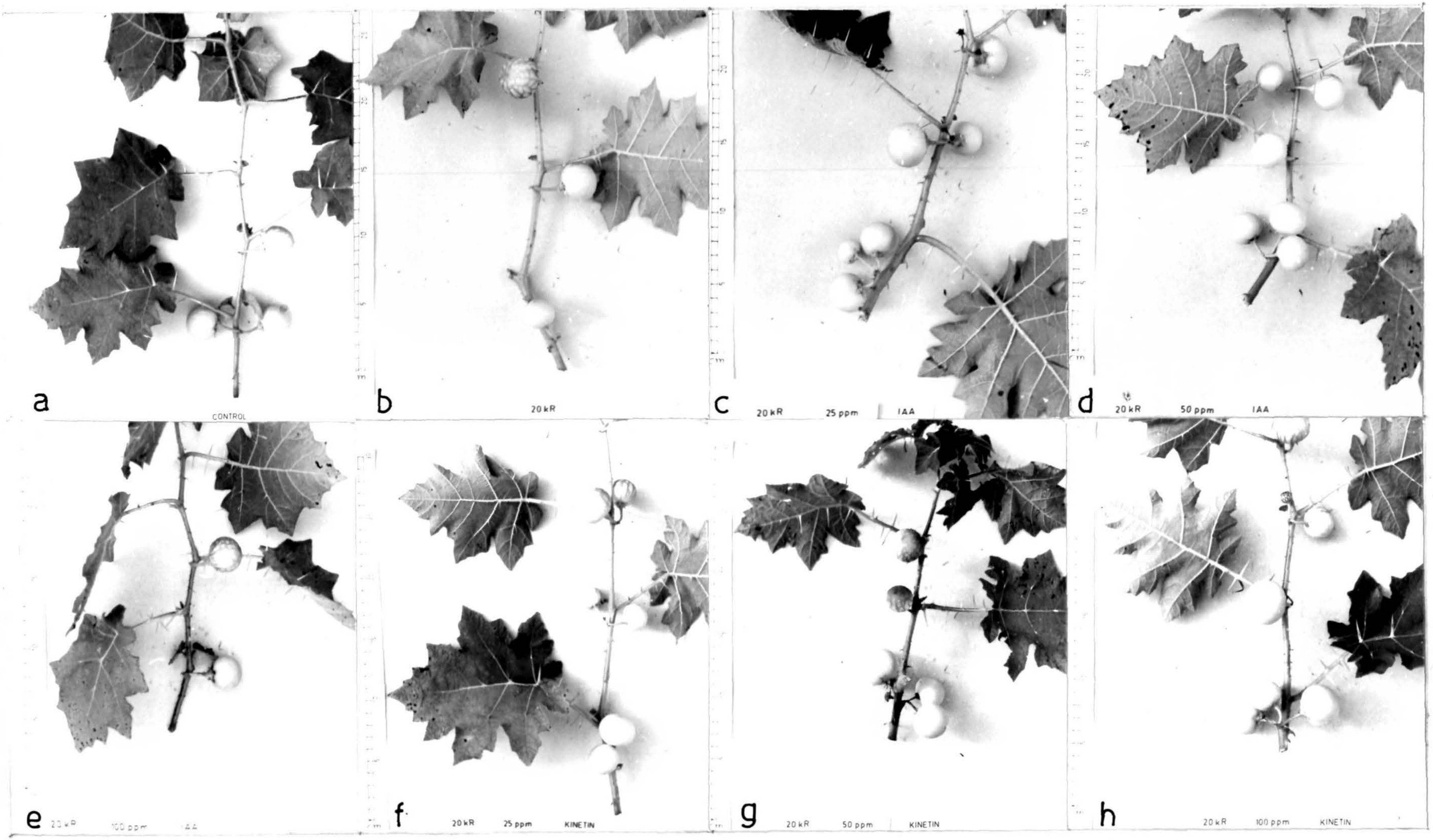

Berry number
SE
Berry number
14
12
10
8
6
4
2
0
Control
20 kR
20 kR + 25 ppm IAA
20k + 50ppm IAA
20kR + 100ppmIAA
20kR + 25ppmK
20kR + 50 ppmK
20kR + 100ppm K
Treatment

Plate 13 : Modulation of radiation effects on berry development in S. khasianum by growth hormones during 1980.

PLATE 13


a
CONTROL
b
20 kR
c
20 kR 25 ppm IAA
d
20 kR 50 ppm IAA
e
20 kR 100 ppm IAA
f
20 kR 25 ppm KINETIN
g
20 kR 50 ppm KINETIN
h
20 kR 100 ppm KINETIN

improved significantly the incidence of long styled flowers over 20 kR plants in the early phase of flowering (Table 32, Fig. 22). These differences, however, became non-significant later (Table 32). The radiation injury to long style production was not influenced by 100 ppm of IAA (Table 32, Fig. 22). Twenty kR plants sprayed with 25 ppm IAA alone produced significantly more berries than irradiated control, other concentrations could not induce significant differences in berry production (Table 33, Fig. 23 and Plate 13 c-e).

Spraying of 20 kR plants with kinetin improved the incidence of long styled flowers. Whereas 25 and 50 ppm kinetin improved the incidence of long styled flowers over 20 kR irradiated plants at the end of fifth spray only, 100 ppm kinetin increased the incidence of long styled flowers from third spray onwards (Table 32, Fig. 22). All the combination treatments though did not differ from control, the differences were significant with reference to 20 kR plants (Table 32). A similar pattern was evident in the berry production per plant (Table 33, Fig. 23 and Plate 13 f-h).

DISCUSSION

I. Effect of age and season on the occurrence of long styled flowers:

Murthy and Abraham (1975a) reported that in *S. khasianum* with an increase in age of the plant the frequency of long styled flowers increased. In the present study, however, the incidence of long styled flowers though increased with the age of the plant in the early phase of flowering (July-August) in the later phase (September-October) it decreased with the progression of age. Since the temperature prevailing in Shillong during September, October was different from the temperature of July, August the possibility of temperature as a factor in the incidence of long styled flowers cannot be ruled out. That temperature is involved in the incidence of long styled flowers is supported from the fact that in this study long styled production reflected the changes in the maximum temperature at Shillong conditions. These observations are in agreement with those of Pal and Singh (1943) in eggplant wherein they have suggested the effect of weather and season to be the main factors for variations in style size. Thus in S. khasianum both age of the plant and temperature influence the expression of heterostyly.

II. Hormonal regulation of heterostyly:

Gibberellic acid cause significant decrease in the incidence of long styled flowers, compared to control. Wakhloo (1975b) also reported enhanced production of female sterile (short styled) flowers in GA_3 treated S. sisymbrifolium plants.

Twentyfive ppm of IAA induced significant increase in the incidence of long styled flowers during early phase of experimentation, but the differences were short-lived. The higher concentrations of IAA (50 and 100 ppm) did not influence the incidence of long styled flowers. Hundred ppm MH, also did not alter significantly the incidence of long styled flowers vis-a-vis control. These findings may therefore suggest that endogenous IAA probably has no direct influence on heterostyly in S. khasianum.

The production of long styled flowers could be under the influence of kinetin. In the present study all the kinetin treated plants had greater incidence of long styled flowers, and the effect persisted throughout the experimentation. The differences vis-a-vis control were, however, significant in plants sprayed with 50 ppm of kinetin. Thus the responses follow an optimum curve. Further, the treatment with Rifampicin significantly inhibited long styled flower incidence and the effect was persistent. The present findings, therefore, suggest the possible involvement of kinetin in the development of long styled flowers in S. khasianum. These conclusions find support from the studies of Wakhloo (1975b) in S. sisymbrifolium where he has shown that kinetin application caused an increase in female fertile (long styled) flowers.

IIIa. Gamma-ray effects on heterostyly:

The incidence of long styled flowers was stimulated by low exposures of gamma rays. The maximum stimulation was noticed in 5 kR irradiated plants and the higher exposures resulted in a slight decrease in the incidence of long styled flowers. Since the effect of gamma-irradiation were reflected on the berry production also, the present study suggests the possibility of increasing berry yield by appropriate exposures. Stimulation of growth, development and yield by gamma rays is reported in literature (Gunckel and Sparrow, 1961; Sax, 1963; Kahan, 1973; Shamsi and Sofajy, 1980).

IIIb. Modulation of radiation responses:

In the preliminary experiments, compared to control there was a significant reduction in long style flower occurrence in 20 kR at the end of second spray. In the later phase the differences became non-significant which could be due to recovery of these plants from radiation injury. Recovery from radiation injury with passage of time is reported in literature (Gunckel and Sparrow, 1961).

GA_3 did not influence radiation responses in the experiment done during 1979 suggesting that GA_3 is not involved in radiation effects on long style development in this species.

Hundred ppm of IAA did not alter radiation effects significantly. Twentyfive and fifty ppm of IAA though induced slight recovery, the effect was very brief. The present findings thus suggest that only low concentrations of auxin are able to reduce radiation-induced inhibition. Similar were the observations of Chauhan (1976), Chauhan and Singh (1975), Chauhan (in press) in *Carthamus tinctorius* and Bhattacharya and Rao (1978) in rice. Post-irradiation modulation of radiation injury by exogenous application of IAA is reported for *Crepis*

(Araratyan and Azatyan, 1974), Soybean (Bhattacharya, 1977) and rice (Bhattacharya and Rao, 1978). Bhattacharya and Rao (1978) are of the opinion that radiation enhances IAA breakdown in irradiated material by stimulating IAA oxidase activity and therefore, exogenous application of auxin restores normal growth in the irradiated plants.

Hundred ppm of kinetin sprays on 20 kR plants resulted in a significant increase in the incidence of long styled flowers in both the experiments. This suggests that exogenous application of kinetin is able to reverse the gamma-ray-induced inhibition of long style development. Thus kinetin functions as a radioprotective agent for this phenonmenon. Radioprotective effects of kinetin are known in *Crepis* (Araratyan *et al.*, 1975) and pea seedlings (Mikhailov *et al.*, 1978). Since in the present study 25 and 50 ppm of kinetin could restore the production of long styles only after repeated sprays, it may be said that for long style formation a minimum threshold level of cytokinin is a necessity. Gamma rays are known to influence cytokinin synthesis in irradiated material (Pandey *et al.*, 1978). Thus it may be visualized that in *S. khasianum* gamma-ray effects on heterostyly (production of long styles) are mediated through its effect on cytokinin metabolism. That this is ao is supported by the fact that exogenous application of kinetin to irradiated plants resulted in restoring the occurrence of long style flowers to normal level.

The present findings thus suggest that the phenomenon of heterostyly in *S. khasianum*, as evident by the production of long style flowers is a complex one and influenced by age of the plant, temperature prevailing and plant hormones. Amongst plant hormones a direct involvement of kinetin is suggested since it influenced the production of long styles in experiment Nos. II and IIIb. The results obtained with Rifampicin further corroborate this conclusion. Since only 25 ppm of IAA influenced production of long styles for a very brief period and as neither exogenous application of MH, an antiauxin, nor IAA in experiment Nos. II and IIIb, respectively, could alter the incidence of long styles, it is assumed that possibly IAA is not involved directly in the production of long stylesin *S. khasianum*. The fact that GA_3 could not modulate radiation effect on long style development and as the incidence of long style was significantly inhibited compared to control only towards the end of preliminary experiment during 1979 it may be concluded that GA_3 effects on long styles are only due to supraoptimal concentration of this hormone. GA_3 also, therefore, does not seem to have any direct role in the production of long styles in this species.

CHAPTER IX

GENERAL DISCUSSION AND CONCLUSIONS

Gamma-irradiation stimulated the rate of germination of seeds in S. khasianum and inhibited this aspect in S. indicum. Patil (1967) reported that in S. khasianum gamma rays delay the rate of seed germination. Pre-sowing gamma-irradiation of seeds markedly improved seed germination in S. khasianum but reduced this in S. indicum, vis-a-vis control. Gamma-ray-induced stimulation and inhibition of germination are reported for other plants also (Gunckel and Sparrow, 1961; Grover and Dhanju, 1979). Bhatt (1972) reported 20 kR as the LD_{50} for seed germination in S. khasianum. Chauhan (1978a) observed that S. khasianum seeds exposed to 25 kR of gamma rays failed to germinate. In the present study, however, exposures upto 30 kR did not inhibit seed germination in this species. On the other hand in S. indicum the LD_{50} for seed germination was between 25 and 30 kR of gamma rays. Thus S. indicum is more radiosensitive than S. khasianum. The differential behaviour of S. khasianum seeds to seed irradiation in the three studies mentioned above could be due to different experimental conditions. Factors like environment, seed moisture content, rate of irradiation etc. are known to alter radiation responses (Gunckel and Sparrow, 1961).

In S. khasianum while, exposures upto 20 kR had no effect on seedling survival, exposures of 25 and 30 kR resulted in a significant decrease in seedling survival. In S. indicum, exposures of 20 kR and above reduced seedling survival drastically, the LD_{50} for seedling survival being between 20 and 25 kR. Thus the minimum exposure required for inducing 50% inhibition to seedling survival differed in the two species with

S. indicum requiring lower exposure of gamma rays. Thus *S. indicum* is more prone to radiation damage than *S. khasianum*. Reduced seedling survival by higher exposures of radiation is reported for other plants also (Fujii and Matsumara, 1958; Micke, 1961; Prasad and Godward, 1975; Torne and Desai, 1975). Findings with *S. khasianum* provide further evidence to the fact that though radiations may not influence seed germination, the seedling survival is drastically reduced.

One of the most characteristic responses of plants to ionizing radiations is inhibition of growth. In general,growth is inversely proportional to the dose rate or total exposure dose of gamma rays administered (Gunckel, 1965). Gamma-ray-induced growth inhibition has been reported for several plant species (Sparrow and Singleton, 1953; Meiselman, 1956; Dumanovic and Ehrenberg, 1965; Mergen and Thielges, 1966; Taylor, 1968; Bajaj *et al.*, 1970; Hussain and Khan, 1971; Rajput, 1971; Chauhan, 1978a;Misra *et al.*, 1979; Khanna and Maherchandani, 1980; Inoue *et al.*, 1980). Stimulation of growth by gamma rays is also reported in literature (Mujeeb and Greig, 1976; Bapat and Rao, 1976; Kothekar, 1977; Shamsi and Bajwa, 1978). In the present study though gamma rays did not influence plant height in *S. khasianum*, significant inhibition of plant height was observed in *S. indicum*. The production of branches was stimulated in *S. khasianum* and inhibited in *S. indicum*. More than 50% reduction in the number of branches per plant was observed in 20 kR plants of *S. indicum*. Thus *S. indicum* is more radiosensitive.

Plastochron index was significantly inhibited by 15 and 20 kR exposures of gamma rays in both the species. The incidence of chimeric leaves and leaf abnormalities in irradiated material were more in *S. indicum* than in *S. khasianum* suggesting greater radiosensitivity of the former species.

In the present study the sixth leaf of the irradiated plants of both the species had less spines compared to control. Relative to *S. khasianum*, inhibition of spine number was more pronounced in *S. indicum*. Thus spine development in *S. indicum* is more radiosensitive. Spine size on the dorsal surface of the sixth leaf in *S. khasianum* was, though not influenced by irradiation, the size of the ventral surface spines was reduced by 15 and 20 kR exposures. On the other hand, in *S. indicum* while 2.5 kR significantly stimulated size of the spines on both the surfaces of the sixth leaf, 20 kR exposure reduced spine size on the ventral surface along. The ventral spines are thus more prone to radiation damage. Similar were the findings of Chauhan (1978a) in *S. khasianum*.

The number of berries produced per plant and the berry yield in the irradiated plants of *S. khasianum* did not appreciably differ from control. All the gamma-ray exposures, however, reduced berry number and berry yield in *S. indicum*. In *S. indicum* more than 50% reduction of berry number and berry yield occurred in 15 and 10 kR exposed plants, respectively. Thus unlike *S. khasianum* berry yield in *S. indicum* is more prone to radiation damage. According to Killion *et al.* (1971)

plant radiosensitivity increases once the plants develop reproductive capabilities. The present findings in *S. indicum* though supports this view, observations with *S. khasianum* did not provide evidence for this. The two species thus differ in their responses to gamma rays.

The glycoalkaloid content in irradiated plants of *S. khasianum* did not differ from control except in 20 kR exposed plants where alkaloid content was more, compared to control. Though 2.5 kR exposure resulted in an increase in glycoalkaloid content in *S. indicum*, the higher exposures were inhibitory. More than 50% reduction in the glycoalkaloid content was found in *S. indicum* in 10 kR exposure. Thus relative to *S. khasianum* this aspect is more drastically influenced in *S. indicum*.

Gamma-ray exposures while influenced topography of apex in *S. indicum*, the outline of the apex did not differ from control in the irradiated *S. khasianum* apices. Vacuolation of tunica and corpus was evident in irradiated apices of both the species. But relative to *S. khasianum* the degree of vacuolation was more in *S. indicum*. Apices of both the species studied have an angiosperm type (Popham, 1952) of cytohistological zonation pattern. Though gamma radiation could not influence cytohistological zonation in *S. khasianum*, in *S. indicum* there was a tendency for disorganization of the zonation pattern in apices of 10-20 kR exposures. In *S. indicum* all the irradiated apices had relatively proximal cell maturation zone compared to control. This aspect was, however, not much influenced in S. khasianum. Excessive cell elongation as a result of radiations are reported in literature (Foard and Haber, 1961; Chauhan, 1976). While irradiated apices of S. indicum had more elongated cells compared to control, in S. khasianum no definite response was evident. Almost all the irradiated apices showed cell disruption and revealed micronuclei in S. indicum. In S. khasianum on the other hand while cell disruption was observed only in 20 kR irradiated apices of 15 days old seedlings, no cell disruption could be noticed in irradiated apices of 60 days old seedlings; suggesting recovery. Micronuclei in S. khasianum irradiated apices were present only in 60 days old seedlings given 5-20 kR exposures. Absence of cytohistological zonation, cell disruption, occurrence of micronuclei, increased vacuolation, excessive cell elongation, acropetal advancement of maturation zone, flattening of apex are considered to be reflections of inhibited meristematic activity (Pratt, 1968; Rai and Singh, 1976). Since these aspects are more pronounced in S. indicum compared to S. khasianum, it may be assumed that S. indicum is more radiosensitive than S. khasianum. These observations find additional support in the growth reactions of 15 and 60 days old seedlings. The present study also provides evidence to suggest that the growth reactions of plants are reflected in responses of shoot apex.

In both S. khasianum and S. indicum only 15 and 20 kR exposures caused an appreciable delay in flowering. Gamma-ray exposures inhibited inflorescence development and flower production more drastically in *S. indicum* than in *S. khasianum*. In *S. khasianum* the various gamma-ray exposures did not elicit any definite response for these aspects. While the number of open flowers per plant in *S. khasianum* was significantly stimulated by 2.5 and 5 kR exposures only in the early phase of flowering, gamma-ray exposures of 10-20 kR induced significant inhibition throughout the season. In *S. indicum* the number of open flowers per plant was inhibited by all the gamma-ray exposures. As this may be a reflection of the radiation effects on inflorescence development and flower production in the species, it may be concluded that the process of inflorescence development and flower formation is more prone to radiation damage in *S. indicum* than in *S. khasianum*. In *S. khasianum* while 5 kR exposure caused an earliness of berry maturity, 15 and 20 kR exposures induced delay. In *S. indicum* on the other hand, though 2.5 kR exposure did not influence berry maturity, all other exposures (5-20 kR) delayed berry maturity, suggesting that radiation effects are more severe in *S. indicum*.

Whereas gamma-ray exposures had no effect on pollen germination in *S. khasianum*, in *S. indicum* the germination percentage decreased with increasing exposures. Pollen tube growth in both the species was inhibited by higher exposures (15 and 20 kR). The inhibition of pollen germination and pollen tube growth induced by gamma rays could be due to gross impairment of binding of calcium ions essential for normal tube elongation and its probable influence on cell membranes and synthesis of cellulose, pectin and callose (Brewbaker and Emery, 1962); effect on cell membrane and enzymes associated with it (Brewbaker *et al.*, 1965); disruption of cell membrane (Pfahler, 1971); cessation of protein synthesis (Cresti *et al.*, 1977) and interference of m-RNA metabolism associated with pollen tube growth (Van der Donk *et al.*, 1978). Thus mechanism of gamma-ray-induced effect on pollen germination and pollen tube growth is complex.

Gamma rays influence heterostyly in *S. khasianum*. While low exposures promoted long style development, the higher exposures favoured development of short styles. The irradiated plants producing predominantly long styled flowers bore greater number of berries. Thus berry yield in this species may be increased by appropriate exposures.

Thus, the data collected on seed germination, seedling survival, plant height, incidence of chimeric and abnormal leaves, spines present on the leaves, number of branches, berry number, berry yield, glycoalkaloid content of berries, production of inflorescence clusters, flowers per plant, number of open flowers per plant and pollen germination of the two species in the present study reveal that gamma-ray exposures caused more injury in *S. indicum* than in *S. khasianum*. This is further supported by observations on shoot apical meristem of the two species.

In the present study gamma-ray exposures stimulated seed germination, number of branches, intensity of spines present on the leaves and number of open flowers per plant in S. khasianum but inhibited these aspects in S. indicum. Gamma-ray exposures had no effect on flower production and inflorescence development in S. khasianum while it inhibited these aspects in S. indicum. Besides, the glycoalkaloid content of S. khasianum was while stimulated by 20 kR exposures, in S. indicum except 2.5 kR all other exposures induced inhibition. Thus the responses of these two species to gamma rays are different. Variation in the response of two species to gamma rays is reported in literature (Gunckel and Sparrow, 1961). 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 effects of a growth hormone in different plants". The differential effects of radiations on different plants can be explained on the above lines since radiations are known to cause hormonal disbalance in irradiated plant material (Skoog, 1934,1935; Siders et al., 1969).

Radiations induce hormonal disbalance in irradiated plant material (Skoog, 1934, 1935; Gordon and Weber, 1955; Siders et al., 1969) and exogenous application of hormones can modulate radiation effects (Gaur and Notani, 1960; Chauhan and Singh, 1975; Chauhan, 1976; Bhattacharya, 1977). Since radiations caused a pronounced inhibition of pollen germination and pollen tube growth in both the species included in the present investigation, it was considered worthwhile to see whether plant growth hormones (GA_3, IAA and kinetin) can modulate radiation responses of pollen grains. It was found that while in *S. indicum* all the concentrations of GA_3 (1, 5, 10, and 25 ppm) induced recovery from radiation injury, in *S. khasianum* concentrations upto 10 ppm were radioprotective but 25 ppm further sensitized pollen germination and pollen tube growth. The toxic effect of 25 ppm GA_3 could be because its concentration may have become supraoptimal. Post-irradiation modulation of radiation injury by GA_3 treatments is reported for maize seedlings (Gaur and Notani, 1960), wheat seedlings (Haber and Luipold, 1960), pinto bean plants (Lockhart, 1961) and *Phaseolus vulgaris* (Silveira and Hell, 1977). All concentrations of IAA in *S. khasianum* and 10 and 25 ppm of IAA in *S. indicum* further radiosensitized the phenomenon of pollen germination and pollen tube growth. However, 1 to 5 ppm of IAA reduced radiation injury to both these aspects in *S. indicum*. Post-irradiation modulation of radiation injury by exogenous supply of IAA is reported for *Crepis* (Araratyan and Azatyan, 1974) soybean (Bhattacharya, 1977) and rice (Bhattacharya and Rao, 1978). Except one ppm of kinetin which helped recover pollen tube growth in both the species, the other concentrations further enhanced radiation injury. Injury to pollen germination was while reduced by 1 ppm kinetin in *S. indicum*, it did not alter radiation injury in *S. khasianum*. Five 10 and 25 ppm of kinetin enhanced radiation injury to both the species. The present findings thus bring out the fact that GA_3 could effectively *counter inhibition of pollen germination and pollen tube growth* induced by gamma rays, IAA and kinetin induced variable responses in germination and tube growth of irradiated pollen.

In *S. khasianum* pollen germination and pollen tube growth were inhibited by GA_3 concentrations, but in *S. indicum* an optimum curve was observed with 5 ppm of GA_3 as the most stimulating concentration. The GA_3-induced stimulation of pollen tube growth is considered to be because of its effect on cell expansion and orientation of newly synthesized cellular microfibrils (Malik and Chhabra, 1976). Most of the concentrations of IAA and kinetin induced inhibition suggesting that the concentrations used were toxic.

The process of heterostyly in *S. khasianum* seems to be under the influence of age, environmental conditions (temperature) and plant growth hormones. It was found that in the present study though in the early phase (July-August) of flowering long style incidence increased with age, in later phase of flowering (September-October) it decreased. Since the environmental conditions prevailing in the two phases of flowering were different it may be assumed that besides age of the plant, temperature also influences long style development. Murthy and Abraham (1975a), who found that long style development increases with increase in age do not, however, mention anything about the role played by fluctuating temperature during the growing season. Involvement of plant hormones in the development of heteromorphic styles in Solanums is suggested (Martin, 1972; Wakhloo, 1975b). An attempt was, therefore made to find out if it is so. It was found that long style development is favoured by kinetin treatments whereas IAA is not directly involved in long style formation. Accumulated GA_3 concentrations reduced the incidence of long styles which could be because of supraoptimal concentration. The present study therefore suggests that instead of IAA (Heslop-Harrison, 1957; Baksh and Iqbal, 1978) it is kinetin which controls the production of flowers with long styles.

Further evidence for kinetin involvement in gamma-ray-induced effects on long style development was obtained while attempting modification of inhibition of long style development induced by gamma rays with growth hormones. It was found that kinetin concentrations (25, 50 and 100 ppm) used in this study could always reduce the injury to long style flowers incidence. Low concentrations of IAA (25 and 50 ppm) though could provide radioprotection for a very brief period it was not effective during most of the time suggesting that radiation effects on long style production are possibly not mediated through its effect on IAA.

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APPENDIX

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GAMMA RAY EFFECTS ON HETEROSTYLY IN *SOLANUM KHASIANUM* CLARKE

Solanum khasianum Cl. yields the alkaloid solasodine, a raw material for the synthesis of Corticosteroids in commercially exploitable amounts[3]. However, attempts to cultivate this species have not been encouraging mainly due to the low berry yield[1]. Murthy and Abraham[4] reported the occurrence of heterostyly in this species. Though they found 90·0% fruit set in long styled flowers, the short styled flowers did not set any fruits. Thus heterostyly may be responsible for poor berry yield of this species. In the present communication the effects of gamma rays on the incidence of heterostyly are described.

Seeds of *S. khasianum* (moisture content 14·5%) were irradiated with 2·5, 5·0, 10·0, 15·0 and 20·0 kR using ^{60}Co source (4·1 kR/min). The irradiated seeds were flown back to Shillong and sown in pots filled with 1 : 1 mixture of soil and farmyard manure. When seedlings were about 75 days old, they were transplanted in the experimental beds (Botanical Garden of the N.E. Hill University). When the plants started flowering, 350–400 flowers were analysed for the incidence of long and short styled flowers in control and in irradiated plants. The average number of berries produced per plant was also recorded for control and irradiated plants.

The per cent occurrence of long and short styles and berry production in both unirradiated and irradiated *S. khasianum* are shown in Fig. 1. The per cent incidence of long and short styled flowers was 43·3 and 56·7 respectively in the control, but the percentage of long styled flowers increased in the irradiated plants. The increased incidence of long styled flowers was also reflected in the enhanced berry production (Fig. 1). Although all the exposures increased the occurrence of long styled flowers and enhanced berry production, the rising trend of the curve was evident up to 5·0 kR only, subsequent to which it showed a decline. Thus, while the per cent incidence of long styled flowers is increasingly stimulated by lower exposures of gamma rays up to a threshold exposure dose, the higher exposures inhibit. Gamma ray exposures have an inverse effect on short styles. Stimulation of growth and development by low exposures of gamma rays and inhibition by higher exposures is reported in literature[2].

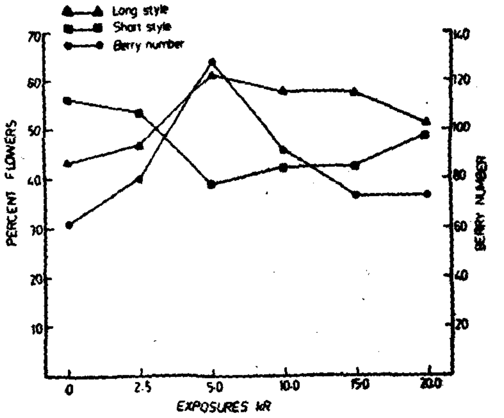

FIG. 1. Effect of γ-ray exposures on the incidence of long and short styles in *S. khasianum* and their relationship with berry formation.

The present study brings out the fact that with appropriate gamma ray exposures, the incidence of long styled flowers can be altered suitably for getting a better berry yield.

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HORMONAL REGULATION OF GAMMA-RAY INDUCED EFFECT ON HETEROSTYLY IN *SOLANUM KHASIANUM* CLARKE

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ABSTRACT

Exogenous application of 100 ppm of IAA and kinetin while do not influence the production of long styled berry forming flowers in *S. khasianum*, GA_3 reduces the production of long styled flowers after repeated sprays. Gamma-ray exposure (20 kR) cause significant injury to the production of long styles. The effect of the above three hormones on modulation of radiation injury was also studied. Whereas, GA_3 and IAA could not modulate radiation-induced inhibition to the production of long styles, kinetin resulted in significant recovery of radiation injury. Thus, kinetin acts as a radioprotector in the present study. The involvement of GA_3 and kinetin in heterostyly in *Solanum khasianum* is conjectured.

Short running title - Chauhan, Y.S. & Ravindran, S. Hormonal regulation of gamma-ray effects on Heterostyly.

Financial assistance from the Council of Scientific and Industrial Research is gratefully acknowledged. We are thankful to Professor P.S. Ramakrishnan, Head of the Department of Botany, North-Eastern Hill University for facilities.

Solanum khasianum Clarke is an important medicinal plant as its berries yield the glycoalkaloid solasodine (Maiti et al. 1964). The berry production in this species is very low mainly due to heterostyly: long style (berry forming) and short style (non-berry forming) flowers (Murthy & Abraham, 1975). Higher exposures of γ-rays inhibited long styles incidence in S. khasianum reducing berry yield (Chauhan & Ravindran, 1979). Martin (1972) suggested that in Solanum mammosum short styled non-berry forming flowers may be produced due to lack of sufficient developmental hormone. Gamma rays affect hormonal balance and bring about changes in growth processes in plants (Gunckel & Sparrow, 1961). At the higher exposures of gamma rays inhibited long style formation, the present study was undertaken to investigate if the radiation-induced effects on heterostyly are mediated through plant hormones and whether radiation effects can be modulated by exogenous supply of the hormone involved. The three hormones used in this study were GA_3, IAA and kinetin.

MATERIALS AND METHODS

The procedure adopted for raising the control and irradiated seedlings are described elsewhere (Chauhan & Ravindran, 1979). Seventyfive day old seedlings of control (unirradiated) and 20 kR S. khasianum were transplanted in pots filled with 1:1 mixture of soil and farmyard manure. The first buds appeared 90 days after sowing. Hormonal sprays were carried out from this stage onwards and were repeated at two week intervals. Hundred ppm each of GA_3 (Gibberellic acid), IAA (Indole-3-acetic acid) and kinetin (6-furfurylaminopurine) were used in the present experiment as foliar spray on control and 20 kR irradiated plants. Care was taken to segregate the treatments and the entire plant was thoroughly doused with the hormonal spray. Control and 20 kR irradiated plants were sprayed only with double glass distilled water. Five plants were maintained per treatment and the treatments were randomized. The observations on the occurrence of long style flowers were carried out at 4 day intervals. The cumulative percentage occurrence of long styled flowers were calculated at the end of each spraying. The flowers began to anthesize only after the second spray. Hence the data has been presented from second spray onwards. The average number of berries produced per plant was recorded for control and the different treatments at maturity.

RESULTS AND DISCUSSION

In control at the end of second spray 43% flowers were long styled and the percent occurrence of long styles decreased with an increase in age of the plant. At the end of fifth spray the percentage incidence of long styled flowers in control plants was 25.7 (Table-1).

Table-1. Effect of growth hormones and γ-ray exposures on percentage incidence of long styled flowers in *Solanum khasianum* (±SE).

TREATMENT	2nd SPRAY	3rd SPRAY	4th SPRAY	5th SPRAY
CONTROL	43.0 ± 7.7	34.0 ± 3.5	28.5 ± 4.6	25.7 ± 4.4
GA_3	24.2 ±14.0	31.9 ± 3.4	17.9 ± 1.1	14.8^{a} ± 1.3
IAA	36.1 ± 5.0	35.7 ± 1.6	24.2 ± 1.1	21.3 ± 1.0
KINETIN	39.3 ± 7.0	38.4 ± 3.3.	29.0 ± 2.1	23.8 ± 1.8
20 kR	23.0^{a} ± 4.0	29.5 ± 4.3	23.4 ± 3.2	20.4 ± 2.2
20 kR + GA_3	25.0 ± 3.5	25.4 ± 2.6	17.6 ± 1.5	15.6 ± 1.3
20 kR + IAA	25.7 ± 1.1	27.6^{c} ± 1.3	20.7^{c} ± 0.7	18.9^{c} ± 1.0
20 kR + KINETIN	51.8 ±^{b} 4.9	43.0 ±^{b} 2.6	36.4+±^{bd} 2.4	31.3 ±^{bd} 2.0

[a] significantly different from control.

[b] significantly different from 20 kR.

[c] significantly different from IAA.

[d] significantly different from kinetin.

Significance tested by 't' test at 0.05 level.

Gibberellic acid treatment reduced the incidence of long styled flowers in the treated plants but the effect significantly differed from control only in the last spray (Table-1).

Both IAA and kinetin treatments though had a tendency to reduce the production of long styled flowers, the differences were never significant vis-a-vis control.

Compared to control, 20 kR irradiated plants produced lesser number of long styled flowers. These differences, which were significant at the end of the second spray, became non-significant in the later stages of development (Table-1). This could be due to the recovery of irradiated plants from radiation-induced injury. Recovery from radiation injury with progress of time from irradiation is reported in literature (Gunckel & Sparrow, 1961; Iqbal, 1970; Lapins & Hough, 1970).

Amongst combination treatments of radiation and hormones 20 kR + GA_3 did not differ significantly from 20 kR irradiated plants (Table-1). In other words, the injury induced by radiation to the production of long styled flowers could not be modified by GA_3 suggesting that GA_3 is neither radiosensitizing nor radioprotecting the phenomenon of heterostyly in S. khasianum. Post irradiation modulation of radiation injury by GA_3 application, however, has been reported in literature (Gaur & Notani, 1960; Haber & Luipold, 1960; Lockhart, 1961; Silveira & Hell, 1977). The combination of 20 kR + IAA did not alter the incidence of long styled flowers vis-a-vis control or plants given 20 kR exposure. However, plants of this treatment did significantly differ from the IAA treated plants in the production of long styled flowers (Table-1). This thus indicates that compared to IAA alone the combination of 20 kR + IAA is more injurious to the production of long styled flowers. Post radiation modulation of radiation injury by exogenous application of auxins has, however, been reported in the literature (Bhattacharya & Rao, 1978; Klein & Klein, 1971).

The combination of 20 kR + kinetin affected the production of long styles favourably (Table-1). While this treatment differed significantly from 20 kR irradiated plants always, the treatment was significant vis-a-vis kinetin treated plants in fourth and fifth sprays (Table-1). The fact that kinetin sprays reduced the gamma ray-induced injury significantly suggest that kinetin acts as a radioprotective agent. Radioprotectiv effects of kinetin are reported in literature (Araratyan et al. 1975; Mikhailov et al., 1978; Jonard et al., 1979).

The effects of different treatments on the incidence of long styles were reflected in the berry production in most of the treatments (Fig.-1).

The present study thus indicates that while IAA and GA_3 did not modulate radiation injury to the formation of long styled flowers in S. khasianum, kinetin helps in the recovery of radiation injury suggesting the possibility of kinetin involvement in radiation-induced effects on the heterostyly in this species (Table-1).

The present study also indicates that GA_3 reduces the incidence of long styles; however, the effect becomes significant only after the fifth spray. Whether GA_3 has a direct or indirect effect on the incidence of heterostyly in S. khasianum, we are not in a position to comment at present. Further, based on the observations with kinetin it may be conjectured that a minimum threshold level of kinetin, which is necessary for the production of long styled flowers, when falls below level adversely affects the production of long styles and favours short styles, as is evident in the irradiated plants. This is confirmed by the finding that expgenous supply of kinetin restores the long style production to normal level. Kinetin in supraphysiological concentration is of no consequence in heterostyly (Table-1). Gamma rays are known to influence cytokinin synthesis in irradiated material (Pandey et al. 1978). IAA does not seem to have any role in the heterostyly.

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STUDIES IN THE REPRODUCTIVE BIOLOGY OF ALKALOID YIELDING SOLANUMS. I. TEMPERATURE, SUCROSE AND BORON REQUIREMENTS FOR POLLEN GERMINATION AND POLLEN TUBE ELONGATION

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ABSTRACT

Temperature, sucrose and boron requirements for pollen germination and pollen tube growth in four Solanum species: *S. aviculare*, *S. laciniatum*, *S. indicum* and *S. khasianum* have been studied. Varying temperature (15 - 30°C) did not affect pollen germination in *S. aviculare* and *S. laciniatum*. But in *S. khasianum* and *S. indicum* the best germination was obtained only at 30°C. The optimum temperature for pollen tube growth was 25°C in *S. aviculare*, *S. laciniatum* and *S. indicum* and 30°C in *S. khasianum*. Pollen of all the species studied required 15% sucrose for maximum germination. However, the optimum sucrose concentration for pollen tube elongation varied with the species viz., *S. aviculare* (10%), *S. laciniatum* and *S. khasianum* (15%) and *S. indicum* (20%). The optimum boron requirement for pollen germination and pollen tube growth was 100 ppm in *S. aviculare* and boron optima for pollen germination and pollen tube elongation. Pollen tube growth in *S. indicum* required a higher boron concentration (300 ppm) than *S. khasianum* (200 ppm).

INTRODUCTION

To facilitate inter-specific and inter-generic hibridization an understanding of the factors controlling pollen germination and pollen tube elongation is essential (Vasil, 1974). Some of the important factors controlling pollen germination and pollen tube elongation are temperature, sucrose and boron and are species specific (Johri and Vasil, 1961). Since information on these aspects of pollen biology is lacking for the different alkaloid yielding _Solanum_ species, an attempt was made to study in vitro the temperature, sucrose and boron requirements for pollen germination and pollen tube growth in _S_. _aviculare_, _S_. _laciniatum_, _S_. _indicum_ and _S_. _khasianum_.

MATERIALS AND METHODS

The pollen grains were germinated on a semisolid sucrose-agar medium. The composition of the basal medium was 10% sucrose, 100 ppm boric acid, 300ppm calcium nitrate, 200 ppm magnesium sulphate, 100 ppm potassium nitrate and 0.6% bacto-agar. The pH of the medium was adjusted 7.3. The effects of varying temperature (15,20,25 and 30°C) on pollen germination and pollen tube growth were studied first using the aforementioned basal medium. In subsequent experiments effects of varying concentrations of sucrose (10, 15 and 20%) and boron (50, 100, and 300 ppm), in the form of boric acid, were studied on pollen germination and pollen tube growth, altering the concentration of one factor (sucrose/boron) at a time in the above basal medium. The inoculated slides were incubated at the optimum temperature of the species, determined earlier.

The just anthesized flowers with undehisced anthers were collected, from plants of various Solanum species growing in the experimental garden of the Botany department, N.E. Hill University, Shillong, between 6.0 - 6.30 a.m. The flowers were brought to the laboratory and the anther tips were cut off with a sharp scalpel. The pollen were inoculated on coverslip, having 0.01 ml of the medium, by gently tapping the anthers. The pollen dusted coverslip was inverted and placed over a metallic ring (thinckness 1.5 mm) prefixed to a glass slide and lined with lanolin. The slides, in all the experiments, were incubated for 4 hr. at the end of which the germinating pollen grains and pollen tubes were fixed by flooding with F.A.A. (5 ml formaldehyde + 5 ml glacial acetic acid + 90 ml 50% ethanol). Four replicate slides were maintained for all the treatments. The number of germinated and ungerminated grains were counted for 5 microscopic fields chosen randomly per slide. Similarly for the pollen tube growth atleast 25 pollen tubes per slide selected randomly, were measured.

RESULTS AND DISCUSSION

Table-1 reveals that in S. aviculare and S. laciniatum varying temperatures (15 - 30°C) did not appreciably affect

Table-1. Effect of temperature on pollen germination and pollen tube growth.

TEMPERATURE (°C)	POLLEN GERMINATION PERCENTAGE				POLLEN TUBE LENGTH (um)			
	15°	20°	25°	30°	15°	20°	25°	30°
S. aviculare	91.2 ± 1.6	86.7 ± 1.9	87.3 ± 1.1	88.2 ± 1.2	123 ± 14	318 ± 8	453 ± 24	353 ± 11
S. laciniatum	75.0 ± 1.6	83.7 ± 1.8	82.6 ± 1	77.4 ± 2	155 ± 5	327 ± 11	341 ± 15	230 ± 14
S. indicum	15.3 ± 2.5	41.2 ± 2.7	36.2 ± 2.1	66.5 ± 1.5	100 ± 10	499 ± 27	607 ± 9	547 ± 11
S. khasianum	29.8 ± 1.8	49.4 ± 2.5	53.3 ± 2.8	70.5 ± 2.3	98 ± 3	206 ± 11	498 ± 12	573 ± 9

pollen germination. In *S. indicum* and *S. khasianum*, however, the best germination was obtained only at 30°C and lower temperature (15 - 25°C) were inhibitory. Pollen tube growth in all these species was considerably affected by varying temperatures. The rate of pollen tube growth increased with increasing temperature upto an optimum, in all the species. While 25°C was the optimum temperature for pollen tube growth in *S. aviculare*, *S. laciniatum* and *S. indicum*, *S. khasianum* required 30°C for maximum pollen tube elongation (Table-1). Such differential temperature requirements have been reported in other plants also (Vasil and Bose, 1959).

Pollen grains of many plants require an optimum concentration of sugar solution for germination (Mukerjee and Das, 1964) which varies from species to species (Johri and Vasil, 1961). Sugars act as nutritive materials for the germination of pollen (Johri and Vasil, 1961) and help maintain a proper osmotic balance between the germinating media and the pollen cytoplasm (Mukerjee and Das, 1964). In the present study pollen germination percentage was maximum in all the species in 15% sucrose (Table-2). These results are in agreement with the sucrose requirements reported for other species (Pfahler, 1967; Seetharam and Kusuma Kumari, 1974). Pollen tube elongation, however, showed a variable response. In the present study while *S. aviculare* required a comparatively lower concentration (10%) for pollen tube growth, the sucrose concentration for maximum pollen tube elongation was rather high in

Table-2. Effect of sucrose on pollen germination and pollen tube growth

SUCROSE CONCENTRATION	POLLEN GERMINATION PERCENTAGE 10%	 15%	 20%	POLLEN TUBE LENGTH (um) 10%	 15%	 20%
S. aviculare	86.8 ± 2.5	90.3 ± 2.2	88.3 ± 2.0	541 ± 35	329 ± 30	252 ± 15
S. laciniatum	60.9 ± 3.7	71.1 ± 1.6	70.0 ± 1.9	258 ± 15	378 ± 17	300 ± 11
S. indicum	59.2 ± 2.9	67.7 ± 1.9	45.2 ± 2.4	314 ± 13	350 ± 19	434 ± 10
S. khasianum	48.1 ± 1.4	58.8 ± 2.1	50.4 ± 2.7	197 ± 7	436 ± 16	167 ± 10

S. indicum (20%). *S. laciniatum* and *S. khasianum*, however, required an intermediate sucrose concentration (15%). Both low and high sucrose requirements for pollen germination and pollen tube growth are reported in literature (Mukherjee and Das, 1964).

Table-3 reveals that in *S. aviculare* and *S. laciniatum* both pollen germination and pollen tube elongation were optimum in the medium containing 100 ppm boric acid. Higher boron concentration resulted in a decrease in pollen germination and pollen tube growth. In *S. khasianum* though the boron requirements for pollen germination was similar to that of *S. laciniatum* and *S. aviculare*, a higher concentration of boron (200 ppm) was required for optimal pollen tube elongation in this species. Thus the pollen germination and pollen tube elongation increased with increasing concentration of boron upto a level subsequent to which it decreased. The level of boron causing inhibition varied with the species (Table 3). Similar results have been reported for other *Solanum* species also (Vasil, 1964). In *S. indicum* both pollen germination and pollen tube elongation were better in the medium containing higher concentrations of boric acid. While best pollen germination was observed in the medium containing 200 ppm boron, the pollen tube elongation was maximum in 300 ppm boron. (Table 3). Higher boron requirements for pollen germination and pollen tube growth are

Table 3: Effect of different concentrations of boric acid on pollen germination and pollen tube growth.

Boric acid concentration	POLLEN GERMINATION PERCENTAGE 50 ppm	100 ppm	200 ppm	300 ppm	POLLEN TUBE LENGTH (um) 50 ppm	100 ppm	200 ppm	300 ppm
S. aviculare	55.7±3.4	87.9±1.4	81.1±2.0	68.7±2.7	191±6	339±16	233±9	171±16
S. laciniatum	63.4±2.5	71.8±2.1	63.0±2.6	56.1±1.3	201±5	291±13	213±12	145±6
S. indicum	36.5±2.8	66.2±3.5	71.0±4.1	68.1±2.9	410±29	526±16	541±11	594±15
S. khasianum	48.0±2.9	60.9±2.4	60.8±2.4	38.6±2.6	126±6	334±16	446±15	110±5

considered to be indicative of lower levels of boron in the plant (Vasil, 1964). The physiological mechanisms associated with boron effect on pollen germination process are rather complex and little understood. However, several functions in the pollen germination are suggested for boron i) the formation of sugar-borate complexes which allow for increased absorption, translocation and metablism of sugars (Vasil, 1964), ii) the synthesis of pectin materials for the wall of actively growing pollen tubes (Stanley and Loewus, 1964), iii) stimulation of chemotropic activity of Ca^{++} (Mascarenhas and Machlis, 1964) and iv) increase oxygen uptake by pollen (O'kelley, 1957). However, since boron effect on pollen tube growth were not closely related to borate effects on respiration or sugar absorption, O'kelley further suggested "boron has a specific role in pollen tube growth, in addition to a stimulatory effect on oxygen uptake and sugar absorption."

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GAMMA-RAY INDUCED VARIATION IN THE DEVELOPMENT AND INJURIES IN THE M_1 GENERATION OF SOLANUM INDICUM L.

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ABSTRACT

Present study deals with γ-ray induced variations on the development and yield of *S. indicum*. Gamma-ray exposures inhibited plant height and plastochron index. Spines on the ventral surface of *S. indicum* leaf were more radiosensitive than dorsal surface spines. While spine size increases in the plants given low exposure of γ-rays, the higher exposures were inhibitory. Lateral branching was stimulated in irradiated plants. Gamma rays inhibited flower clusters, berry number, berry weight, berry yield and seed number per berry; of these berry yield was most sensitive to γ-irradiation. Solasodine production was increased by low exposure but higher exposures were inhibitory.

INTRODUCTION

The glycoalkaloids of Solanum species have assumed great commercial importance in recent years on account of their utility as substitutes for diosgenin in the synthesis of steroid hormones. The importance of Solanum indicum as an alternate source of solasodine is known (Chopra and Kapoor, 1968). But this species needs genetic upgrading for higher yield before commercial cultivation is feasible. In recent years gamma rays have been extensively used to induce mutation and increasing genetic variability in diverse medicinal plants (Bhatt, 1972; Chauhan et al. 1976). However, no attempt has been made as yet to improve S. indicum using radiation as a tool. The mutagenic efficiency is determined to a considerable extent by the degree of injuries of M_1 generation (Ehrenberg, 1971) and such injuries also affect the size of M_1 population to be treated and the M_2 to be raised (Blixt, 1972). Since this information is not available for S. indicum an attempt was made to analyse the effect of acute exposures of gamma rays on plant development and γ-ray induced injury in the M_1 plants of this species.

MATERIALS AND METHODS

Dry seeds of S. indicum (moisture content 14.17%) were irradiated at Bhabha Atomic Research Centre, Trombay with the help of a ^{60}Co source emitting 4.1 kR/minute. The acute gamma-ray exposures used were 2,5, 5.0, 10.0, 15.0 and 20.0 kR. The irradiated seeds were flown back to Shillong and sown in pots filled with 1:1 mixture of soil and farmyard manure. Per treatment 250 seeds were sown at the rate of 50 seeds/pot. The pots were watered regularly to maintain sufficient moisture for germination.

Two and a half months old seedlings were transplanted in the experimental plots of North-Eastern Hill University, Shillong. Observations on the growth and reproductive characters of control and irradiated plants were recorded from 10 plants per treatment. Since initiation of flowering occurs both in control and irradiated plants approximately 90 days after sowing, the following growth characters were recorded in 90 days old seedlings; plant height, plastochron index, maximum length and breadth of the 6th leaf. The leaf area was calculated by multiplying length with breadth. Spine number and size on the dorsal and ventral surfaces of the 6th leaf was also recorded. Spine frequency (intensity) per unit area was calculated by dividing spine number with leaf area. Spine size was determined by measuring all the spines from dorsal and ventral surfaces of the 6th leaf. The plants were harvested 9 months after sowing and the various vegetative and reproductive characters considered were, number of branches, flower clusters, berry number, berry yield per plant. The average berry weight and seed number per berry were determined from 20 batches per treatment of 5 berries each.

Table 1: Effect of gamma-ray exposures on growth of *S. indicum* 90 days after sowing.

Treatment	Height cms	Plastochron index
Control	4.9 ± 0.35	8.7 ± 0.40
2.5 kR	5.2 + 0.27 (+ 6.12)	8.6 + 0.42 (- 1.14)
5.0 kR	3.7*+ 0.14 (- 24.48)	8.3 + 0.26 (- 4.59)
10.0 kR	4.1*+0.18 (- 16.32)	7.8 + 0.29 (- 10.34)
15.0 kR	3.2*+ 0.24 (- 34.69)	7.5*+ 0.20 (- 13.79)
20.0 kR	2.6*+ 0.22 (- 46.93)	6.2*+ 0.43 (- 28.73)
C.D. at 5% level	0.65	0.93

Figures in bracket denote percent inhibition (-)/stimulation over control (+).

Solasodine content on a percentage dry weight basis was determined from 3 replicates per treatment following the method of Crusena et al. (1965).

Percentage of inhibition or stimulation was calculated with the help of the formula:

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

RESULTS AND DISCUSSION

Table-1 reveals that though gamma-ray exposures of 5 kR and above significantly inhibited plant height, only 15 and 20 kR exposures could inhibit plastochron index. Growth inhibition by gamma irradiation in solanaceous species has been reported in *Lycopersicon* (Sparrow and Singleton, 1953), *Nicotiana* (Mieselman, 1956) and *S. khasianum* (Chauhan, 1978). Radiation induced reduction in seedling height and growth inhibition may be due to destruction or damage to apical meristems (Patel and Shah, 1974), partial failure of the internodes to elongate, decrease in the number of proliferating cells (Van't Hof and Sparrow, 1965), chromosome structural damage in meristematic cells following irradiation (Gray and Scholes, 1951) and auxins (Skoog, 1935). However, according to Sparrow et al. (1956) the ascribing of reduced stem elongation to reduced auxin levels alone may be an oversimplification since nutritional levels and

Table 2: Effect of gamma-ray exposures on the 6th leaf of *S. indicum* 90 days after sowing.

Treatment	Length	Breadth	Area (cm)^2	Average spine number	Spine intensity/ area	Spine size
Control	4.9 ± 0.6	3.9 ± 0.5	19.1	8.2	0.42	4.5
2.5 kR	5.7 ± 0.2 (+ 16.32)	5.1*±0.3 (+ 30.76)	29.0 (+ 51.83)	6.4 (- 21.95)	0.22 (- 47.61)	6.2 (+ 37.77)
5.0 kR	4.9 ± 0.1 (0.0)	4.3 ± 0.2 (+ 10.25)	21.0 (- 9.94)	5.0 (- 39.02)	0.23 (- 45.23)	5.8 (+ 28.88)
10.0 kR	5.2 ± 0.2 (+ 6.12)	4.5 ± 0.2 (+ 15.38)	23.4 (+ 22.51)	5.1 (- 37.80)	0.21 (- 50.0)	5.2 (+ 15.55)
15.0 kR	4.0 ± 0.3 (- 18.36)	3.6 ± 0.3 (- 7.69)	14.4 (- 24.60)	2.8 (- 65.85)	0.19 (- 54.76)	4.6 (+ 2.22)
20.0 kR	3.7*± 0.1 (- 24.48)	3.3 ± 0.2 (- 15.38)	12.2 (- 36.12)	2.1 (- 74.39)	0.17 (- 59.52)	3.0 (- 33.33)
C.D. at 5% level	1.02	1.00				

Figures in bracket denote percent inhibition (-)/stimulation over control (+).

mechanisms of assimilation may also be important factors.

Effect of γ-ray exposures on leaf expansion in the 6th leaf was also studied (Table-2). While low exposures of γ-rays did not affect leaf length in irradiated plants, only 20 kR exposure caused significant inhibition, compared to control. The lateral expansion of the 6th leaf was significantly stimulated in 2.5 kR exposure and the higher exposures had no effect on lateral expansion of leaf. Thus here leaf length was more affected by γ rays than leaf breadth. This could be due to greater injury to the leaf tip meristem compared to the plate meristem (Table-2). Similar were the observations of Chauhan (1978) in *S. khasianum*.

Leaf area was slightly stimulated by low exposures (2.5, 5.0 and 10 kR) but the higher exposures (15 and 20 kR) were inhibitory (Table-2). Inhibition of leaf expansion by γ-rays has been reported in *Solanum khasianum* (Chauhan, 1978) and other plants also (Gunckel and Sparrow, 1961).

Spine number and spine intensity/area showed a decrease with an increase in γ-ray exposures (Table-2). The reduction in spine intensity is due to reduction in spine number. In *S. khasianum*, however, spine intensity increased with an increase in exposure (Chauhan, 1978). The difference in the two species is because while in *S. khasianum*, compared to spine number, more injury was caused to leaf expansion, in *S. indicum* it is the

Table 3: Gamma-ray effect on the 6th leaf (dorsal and ventral surfaces) of *S. indicum* 90 days after sowing

Treatment	Dorsal surface			Ventral surface		
	Spine number	Spine frequency	Spine size	Spine number	Spine frequency	Spine size
Control	10.5 ± 1.0	0.54	4.7 ± 0.2	5.9 ± 0.8	0.30	4.4 ± 0.3
2.5 kR	8.6 ± 0.9 (- 18.09)	0.29 (- 46.29)	6.4* ± 0.3 (+ 36.17)	4.2* ± 0.5 (- 28.81)	0.14 (- 53.33)	6.1* ± 0.3 (+ 38.63)
5.0 kR	6.9* ± 0.6 (- 34.28)	0.32 (- 40.74)	5.9 ± 0.4 (+ 25.53)	3.2* ± 0.4 (- 45.76)	0.15 (- 50.0)	5.8* ± 0.4 (+ 31.81)
10.0 kR	7.2 ± 0.7 (31.42)	0.30 (- 44.44)	5.5 ± 0.4 (+ 17.02)	3.1* ± 0.5 (- 47.45)	0.13 (- 56.66)	5.0 ± 0.4 (+ 13.63)
15.0 kR	3.8* ± 0.7 (- 63.80)	0.26 (- 51.85)	4.8 ± 0.6 (+ 2.12)	1.9* ± 0.3 (- 67.69)	0.13 (- 56.66)	4.4 ± 0.6 (0.0)
20.0 kR	3.3* ± 0.7 (- 68.57)	0.27 (-50.0)	3.7 ± 0.5 (- 21.27)	0.9* ± 0.2 (- 84.74)	0.07 (- 76.66)	2.4* ± 0.5 (- 45.45)
C.D. at 5% level	3.69		1.24	1.53		1.38

Figures in bracket denote percent inhibition (-)/stimulation over control (+).

spine number which is affected more. Though leaf expansion is stimulated by the lower exposures, higher exposures cause very little inhibition. Variation in the response of the different species are reported in literature (Gunckel and Sparrow, 1961). Wareing (1977) while discussing the effect of growth hormones on integration in higher plants emphasized "the specificity of the response to a given growth substance is usually determined by 'Competence' or programming of the target tissue and which may be the reason for different effects of a growth hormone in different plants". The differential effects of radiations on different plants can be explained on the above lines since radiation are known to cause hormonal disbalance in the irradiated plant material (Skoog, 1935; Siders et al., 1969).

Analysis of spines on dorsal and ventral surface of the 6th leaf revealed that more spines are produced on the dorsal surface compared to the ventral surface (Table-3). Spine intensity on both the surfaces showed a decrease with an increase in γ-ray exposure. The injury caused to spine number and spine intensity, compared to dorsal surface, was more on the ventral surface suggesting that spines of this side were more radiosensitive. Spine size on both the surfaces were stimulated significantly by low exposures of gamma rays (2.5 kR) and inhibited by high exposures (20 kR). Inhibition of spine size by high exposures is reported for S. khasianum also (Chauhan, 1978).

Table 4: Effect of gamma rays on the yield and solasodine content in *S. indicum*.

Treatment	No. of branches	Flower Clusters	Berry No. Per plant	Berry weight gms	Beryy yield gms	No. of seeds per befry	Solasodine % (on dry wt. basis)
Control	50 ± 5.4	52 ± 6.4	78 ± 18.6	0.541 ± 0.02	41.4 ± 9.8	41.± 1	0.29 ± 0.025
2.5 kR	66*±6.2 (+ 32.0)	54 ± 5.8 (+ 3.84)	56 ± 11.7 (- 28.20)	0.539 ± 0.023 (- 0.36)	29.5 ± 6.1 (- 28.74)	36*± 1.7 (- 11.13)	0.45*± 0.039 (+ 55.17)
5.0 kR	69*±6.1 (+ 38.0)	53 ± 4.1 (+ 1.92)	77.±13.3 (- 1.28)	0.485*± 0.018 (-10.35)	37.7 ± 6.6 (- 8.93)	33*± 1.6 (- 19.12)	0.27 ± 0.026 (- 6.89)
10.0 kR	63 ± 3.8 (+ 26.0)	42 ± 4.3 (- 19.23)	45 ± 10.2 (- 42.30)	0.385*±0.018 (- 28.83)	17.9*±4.0 (- 56.76)	24*± 1.6 (- 39.95)	0.11*± 0.011 (- 62.06)
15.0 kR	53 ± 5.7 (+ 6.0)	40 ± 5.0 (- 23.07)	37 ± 11.5 (- 52.56)	0.396*±0.024 (- 26.80)	15.6*±4.8 (- 62.31)	30*± 2.3 (- 26.39)	0.10*± 0.011 (- 65.51)
20.0 kR	44 ± 3.0 (- 12.0)	29*± 5.0 (- 44.23)	23*± 4.7 (- 70.51)	0.300*± 0.026 (- 44.54)	8.2*± 1.7 (- 80.19)	22*± 1.9 (- 46.48)	0.08*± 0.009 (- 72.41)
C.D. at 5% level	14.19	14.38	34.1	0.06	16.75	4.88	0.065

Figure within bracketsdenote percent inhibition (-)/stimulation (+) over control.

In the present study while lower exposures of γ rays induced significant increase in the number of branches, the higher exposures did not inhibit number of branches formed (Table-4). Ionizing radiations are known to break apical dominance and accelerate lateral branching (Sax, 1961). According to Skoog (1935), relatively small doses of x-rays reduce or inactivate auxin production, and development of lateral buds are promoted by irradiating shoot tips. Radiation induced stimulation of side shoots is reported in literature (Kahan, 1973).

Flower clusters, berry number, berry weight, berry yield and seed number per berry were inhibited by γ-ray exposures. Of these berry yield was most sensitive to γ-radiation (Table-4). Killion et al. (1971) reported greater sensitivity of yield compared to stem length and observed that plant radiosensitivty increases once hthe plant develops reproductive capabilities. The present study psovides an evidence in this direction. The reduction in berry yield is due to a reduction in berry number and berry weight. In S. *khasianum*, however, decrease in yield was due mainly to reduction in berry weight (Chauhan, 1978). Radiation-induced reduction in yield is reported for many plants (Davies, 1973; Pautrier, 1976).

Solasodine percentage showed a slight increase in 2.5 kR as compared to control and the difference was significant. The higher exposures, however, resulted in a decrease in the glycoalkaloid content.(Table-4). Radiation-induced increase of alkaloid synthesis is reported for Datura metel also (Kaul et al. 1973).

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*Not seen in original.