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Summary  The present investigation deals with the effects of γ-radiation (5–500 Gy) and growth hormones (IAA, GA and kinetin, 10, 25 and 50 ppm) on pollen germination and pollen tube elongation in *P. kesiya* Royle ex Gord. The effects of combination treatments of γ-rays (500 Gy) and 10 and 50 ppm of growth hormones (IAA, GA and kinetin) were also investigated to find out if radiation effects could be modified by growth hormones. The study revealed that 7.5–40 Gy γ-rays stimulated pollen germination and tube growth, but radiation doses 50–500 Gy were inhibitory. Development of longer pollen tubes was favored by lower doses of γ-rays while higher doses favored development of smaller pollen tubes. 10 ppm of IAA and GA promoted both pollen germination and pollen tube elongation, 25 and 50 ppm of IAA and GA and all the concentrations of kinetin (10–50 ppm) inhibited pollen germination and tube growth. In combination treatments 10 ppm of IAA and GA could ameliorate radiation injury but 25 and 50 ppm of IAA and GA as also 10–50 ppm of kinetin further enhanced radiation damage. Thus in the present study 10 ppm of IAA and GA were radioprotective while 25 and 50 ppm of IAA and GA and 10–50 ppm of kinetin functioned as radiosensitiser.

Pinene rich turpentine oil (Singh and Mehra 1977) of *Pinus kesiya* is a major source of raw material for certain organic compounds. However, the species suffers from low yield of turpentine (Chadha 1977). The pine species have limited genetical variation and poor hybridization possibilities (Rudolph 1972). Irradiated pollen have been used for inducing variation and overcoming hybridization barrier in plants (Rudolph 1978). However, for successful breeding programme based on irradiated pollen the understanding of pollen radiobiology becomes pertinent. Ionizing radiation (Zelles 1974, Chauhan and Katiyar 1990) and plant growth hormones (Dhawan and Malik 1981) influence the germination of pollen and tube growth. Radiation responses can be modulated by exogenous application of growth hormones (Klein and Klein 1971). Almost no information is available on the modulatory effects of growth hormones on irradiated pollen. Therefore, the present study deals with the effects of radiation and growth hormones (IAA, GA and Kinetin) individually and in combination with radiation doses on germination of pollen and tube growth in *P. kesiya*.

Material and methods

Pollen of *P. kesiya* were collected from the trees growing in Shillong and stored in a deep-freeze at −5°C. Equal amounts of the stored pollen was taken in small corning glass-tubes 7.5 cm×0.5 cm. These tubes, containing pollen, were irradiated with 5, 7.5, 10, 20, 30, 40, 50, 100, 200, 300 and 500 Gy radiation doses in a γ-chamber 900 having a 60Co radiation source and emitting γ-rays at the rate of 0.74 Gy/sec. Unirradiated pollen served as control. Both control and irradiated pollen were germinated on a basal medium containing 0.03% sucrose, 5 ppm boric acid and having pH 7.3 (Katiyar and Chauhan 1988).

Effects of growth hormones on pollen germination and tube growth were studied by germinat-
ing control and irradiated pollen on the basal medium supplemented with different concentrations of various growth hormones (GA₃, IAA and Kinetin). Only one concentration (10/25/50 ppm) of a growth hormone was incorporated in the basal medium at a time and the pH of the medium was adjusted always to 7.3 using 1 N NaOH/HCl (Chauhan and Katiyar 1988).

The modifying effects of growth hormones on radiation responses of irradiated *P. kesiya* pollen were investigated by germinating irradiated (500 Gy) pollen in the basal medium supplemented with the growth hormones mentioned above.

To investigate effects of various treatments on pollen germination and pollen tube elongation, unirradiated (control) and irradiated (5–500 Gy) pollen were dusted separately on coverslips, having a drop (0.05 ml) of pollen germinating medium. The pollen-dusted coverslip was inverted and placed over a metallic ring (3.0 mm thickness) prefixed to a glass slide. The rim of the ring was smeared with petroleum jelly prior to placing of the pollen dusted coverslip. The pollen grains were incubated in dark in an incubator maintained at 25±1°C. At the expiry of incubation period (48 hr), the pollen and tubes were fixed with a drop of FAA (90 ml 50% ethanol + 5 ml glacial acetic acid + 5 ml formaldehyde) for further observations and recording of the data on pollen germination and tube elongation. Per treatment, five slides were maintained and data on pollen germination were recorded by counting germinated and ungerminated pollen from 5 microscopic fields, chosen randomly, per slide. For every treatment 25 pollen tubes were measured per slide to study the effects of treatments on tube elongation. Thus for any given treatment a total of 125 tubes were measured. The data were analyzed statistically.

Results and discussion

**Radiation**

At the end of 48 hr incubation 59.01% of control pollen germinated and pollen tubes measured 73.6 μm. Radiation dose 5 Gy did not affect pollen germination and tube elongation (Table 1). But radiation doses 7.5–500 Gy influenced both the processes (Table 1). Radiation doses 7.5–40 Gy stimulated germination of pollen; 30 Gy being the optimal dose. Radiation doses 50 Gy onwards, however, inhibited germination. The inhibition increased with the increasing dose. The radiation effects on plants could be grouped into three categories: (a) low dose irradiation usually having neither stimulatory nor inhibitory effect, (b) sub-lethal doses causing either inhibitory or stimulatory effects on various biochemical and biological processes and (c) high doses of radiation inducing maximum damage and lethality (Seibold et al. 1979). In this investigation 5 Gy radiation dose therefore belongs to the (a) category while 7.5–40 Gy doses are of category (b) and doses 50 Gy and above correspond to (c) category of Seibold *et al.* (1979), respectively. In radiation investigation pollen is considered a multitarget system (Speranza *et al.* 1982). Therefore, for manifestation of radiation caused lethal effects inactivation of more than one target (key molecules, cellular components etc.) would be required (Arena 1971, Speranza *et al.* 1982). This may be the reason for high degree of radiosensitivity of *P. kesiya* pollen. Where as radiation doses 10–50 Gy stimulated tube growth 100–500 Gy caused inhibition (Table 1). Radiation-induced stimulation and inhibition of

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±S.E. *Significantly different from 0 Gy (p=0.05).
Table 2. Effect of radiation on pollen tube size in *P. kesiyana*

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tube elongation are reported in *Pinus patula, P. wallichiana*, Douglas fir, *Nicotiana tabacum, Cucumis* sp., maize, apple *etc.* (Katiyar and Chauhan 1987a, b, Livingston and Stettler 1973, Michie and Bohm 1989, Cuny and Roudot 1991, Dennisen and Den Nijs 1987, Pfahler 1971, Speranza et al. 1982). Stimulatory effect of radiation are still ill-understood (Michie and Bohm 1989). Livingston and Stettler (1973) stated that radiation evoked stimulation of tube elongation is due to its effect on pollen metabolism. In *Pinus*, radiation stimulated pollen tubes by influencing RNA synthesis (Zelles 1974). Radiation-induced stimulation of pollen tube elongation in Douglas fir is due to the de- and re-masking of mRNA (Van der Donk et al. 1978). In Douglas fir radiation in irradiated pollen tubes augments availability of messengers for translation resulting in more protein synthesis and thus higher metabolic activity, compared to control. This increased metabolic activity may be the reason for stimulation of tube elongation. Conversely, radiation-induced inhibition of tube elongation is the indication of damaged cell membrane (Brewbaker and Emery 1962, Pfahler 1971, Visser and Oost 1981). Compared to control, the frequency of relatively long pollen tubes was more in pollen given 10–50 Gy radiation. Pollen given 20 Gy γ-rays developed longest pollen tubes (Table 2). But pollen irradiated with 100–500 Gy γ-rays developed more number of small pollen tubes (Table 2). Development of short pollen tubes due to sublethal doses of radiation are attributed to the impairment in the binding of calcium ion essential for normal tube elongation (Brewbaker and Emery 1962, Speranza et al. 1982). Therefore, it may be assumed that the radiation doses that cause development of long pollen tubes possibly enhance the binding of the calcium. However, low incidence of pollen tubes measuring 81–96 μm at all the radiation doses in the present study was enigmatic (Table 2).

### Growth hormones

The control pollen, at the end of 48 hr incubation, revealed 55.46% germination and tubes measured 77.95 μm in length. Both IAA and GA (10 ppm) promoted pollen germination and tube elongation compared to control (Table 3). The degree of stimulation of tube growth was more in GA treated tubes than IAA treated ones. 25 and 50 ppm of IAA and GA inhibited both pollen germination and tube elongation over control (Table 3); inhibition being more at higher concentration. Compared to control, pollen treated with 10 ppm IAA or GA developed more number of long pollen tubes (Table 4). The higher concentrations of both IAA and GA reduced pollen tube elongation and thus favored development of smaller pollen tubes (Table 4). Exogenous supply of GA affects pollen tube elongation by influencing cell expansion and orientation of newly synthesised mi-

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<th>Unirradiated Pollen tube (μm)</th>
<th>500 Gy irradiated Germination (%)</th>
<th>500 Gy irradiated Pollen tube (μm)</th>
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*± S.E. * Significantly different from control at p=0.05.
Table 4. Effect of growth hormones on pollen tube size in *P. kesiya*

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Table 5. Effect of growth hormones on pollen tube size in 500 Gy irradiated pollen of *P. kesiya*

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<td>30±5</td>
<td>10±2</td>
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<td>51±2</td>
<td>22±2</td>
<td>3±1</td>
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<tr>
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<td>—</td>
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<tr>
<td>GA3 10 ppm</td>
<td>—</td>
<td>—</td>
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<td>22±5</td>
<td>22±2</td>
<td>30±3</td>
<td>13±2</td>
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<td>—</td>
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<td>48±2</td>
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<tr>
<td>GA3 50 ppm</td>
<td>—</td>
<td>—</td>
<td>42±3</td>
<td>46±2</td>
<td>12±2</td>
<td>—</td>
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<tr>
<td>Kin. 10 ppm</td>
<td>—</td>
<td>—</td>
<td>62±7</td>
<td>38±2</td>
<td>—</td>
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<tr>
<td>Kin. 25 ppm</td>
<td>—</td>
<td>—</td>
<td>24±2</td>
<td>64±3</td>
<td>12±2</td>
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<td>Kin. 50 ppm</td>
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<td>41±2</td>
<td>58±2</td>
<td>1±0</td>
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crofibrils in the pollen tube wall (Malik and Chhabra 1976). Pollen contain sufficient amount of auxin for optimal growth in vitro and only under suboptimal conditions exogenous supply of auxin improves tube elongation (Brewbaker and Majumdar 1961). This could be the reason why 10 ppm of IAA and GA induced stimulation and 25 and 50 ppm of IAA and GA caused inhibition of tube elongation. All the concentrations of kinetin inhibited pollen germination and tube elongation vis-a-vis control (Table 3). Thus IAA, GA and kinetin influence pollen germination and tube elongation in *P. kesiya* (Table 3). Besides, the kinetin-induced responses of *P. kesiya* pollen are different from the responses of IAA and GA suggesting that pollens of a species respond differently to different growth hormones (Setia et al. 1985). Wareing (1977) stated that distinctive response of different species to a given growth substance is determined by the ‘competence’ or programming of the target tissue. This may also be the reason why different growth substances elicit dissimilar responses in *P. kesiya*.

**Radiation + growth hormones**

Compared to unirradiated (control) pollen of *P. kesiya*, 500 Gy irradiated pollen exhibited inhibition of germination and tube growth (Table 3). When 500 Gy irradiated pollens were germinated on medium supplemented with 10 ppm of IAA or GA the radiation-induced inhibition of pollen germination and tube elongation was greatly ameliorated (Table 3). Higher concentrations of IAA and GA (25, 50 ppm), however, further accentuated radiation-induced inhibition of pollen germination and tube elongation (Table 3). Frequency of relatively long pollen tubes was more in 500 Gy + 10 ppm GA and 500 Gy + 10 ppm IAA treated pollen (Table 5). But higher concentrations of GA and IAA (25, 50 ppm) favored development of short pollen tubes in 500 Gy irradiated pollen. The frequency of small pollen tubes increased with the increasing concentration of GA/IAA (Table 5). Kinetin (10–50 ppm) increased radiation-induced inhibition of pollen germination and tube elongation (Table 3). Thus where as lower concentration (10 ppm) of IAA and GA ameliorated radiation damage, all the concentrations of kinetin (10–50 ppm) and higher concentrations (25, 50 ppm) of IAA and GA further accentuated radiation damage. Radioprotective and radiosensitising effects of growth hormones are reported in *Crepis capillaris*, *Carthamus tinctorius*, maize, pea etc. (Araratyan et al. 1975, Chauhan 1980, Gaur and Notani 1963, Klein and Klein 1971, Mikhaliov et al. 1980). Ionizing radiations affect endogenous auxin content in the treated plants (Gordon 1957). Auxin content of the test material is an important factor in chemical radioprotection (Skoog 1934). Most of the pollen grains contain sufficient auxin for optimal growth in vitro (Linskens and Kroh 1970). Exogenous supply of auxin stimulates pollen tube elongation only if auxin concentration is suboptimal (Brewbaker and Majumdar 1961). The radioprotective effects of lower concentration of IAA (10 ppm) observed may be because of radiation effect on the auxin content of *P. kesiya* pollen. Further accentuation of radiation damage by higher concentrations of IAA (25, 50 ppm) could be because these concentrations were supraoptimal. Cresti et al. (1977) stated that in *Lycopersicum peruvianum* radiation inhibited pollen tubes reveal cisternae of rough endoplasmic reticulum as whorls of concentric circles (CER) that are indicative of cessation of protein synthesis. Further, application of GA3 to temporarily inactive cells of potato tubers simultaneously induce metabolic activity and the disappearance of CER (Shih and Rappaport 1971). Thus amelioration of radiation damage elicited by lower concentrations of GA may be due to its effect on the metabolism of irradiated tubes. All the concentrations of kinetin further accentuated radiation damage suggesting that possibly the concentrations used (10–50 ppm) were toxic being supraoptimal in nature.

**References**

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1998


