EFFECTS OF GROWTH HORMONES AND GAMMA RADIATION ON POLLEN GERMINATION AND POLLEN TUBE GROWTH OF *SOLANUM MARGINATUM* L.f.

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Abstract

The present study deals with the effects of growth hormones (Kinetin, IAA and GA_3) and gamma rays (1, 50, 100, 200, 500 and 800 krad) on pollen germination and pollen tube growth of *Solanum marginatum* L.f., an alkaloid yielding species. Kinetin (1-25 ppm) and IAA (5-25 ppm) inhibited both pollen germination and pollen tube elongation. One ppm of IAA inhibited the pollen germination but the pollen tube elongation was not influenced. Similarly, concentrations of GA_3 (1-25 ppm) also inhibited germination of *S. marginatum* pollen without any effect on the pollen tube elongation. The pollen germination was stimulated by 200 krad and inhibited by 500 and 800 krad. LD_{100} dose for pollen germination was 800 krad, while LD_{50} was 500 krad. Pollen tube elongation at 1, 50 and 100 krad doses of radiation remains unaffected.

Introduction

Berries of *Solanum marginatum* contain solasodine which is used in the production of steroid hormones in commercially exploitable amounts (Cruz & Proano, 1970). However, commercial production of berries is hampered due to presence of sharp spines, asynchronous flowering and low berry yield. Attempts to improve some species of *Solanum* have not succeeded.

Induced mutations are considered an alternative to hybridization and genetic recombinations in plant breeding. Many medicinal and aromatic plants have been improved by inducing mutations with gamma rays (Kapoor & Datta, 1967) but attempts to improve *S. khasianum* have met with limited success (Bhatt, 1972; Chauhan *et al.*, 1975).

Stair and Mergen (1964) suggested that pollen irradiated with low level of radiation could be used to obtain mutation. Pfahler (1983) emphasized that the gametophytic selection is more effective than sporophytic selection in plant breeding. A great advantage of the pollen irradiation method as opposed to the irradiation of seeds for growing plants is the fact that the former rarely produces chimeras (Briggs, 1970). However, for the success of plant breeding programme employing radiated pollen the first pre-requisite is to understand radiobiology of the species concerned since great differences exist in the radiosensitivity of different species (Sparrow *et al.*,1961).

The understanding of the factors controlling pollen germination and pollen tube growth is also a pre-requisite for a successful hybridization programme (Vasil, 1964). Besides temperature, sucrose, boron, calcium, magnesium and potassium, growth hormones are also considered to be important factors which affect pollen germination and pollen tube growth (Johri *et al*, 1977). However, these requirements are species specific (Johri & Vasil, 1961). Hence this study was conducted to investigate the effects of growth hormones (Kinetin, IAA and GA_3) and gamma radiation on pollen germination and pollen tube growth of *S. marginatum.*

Material and methods

The pollen grains were germinated on a semi-solid sucrose-agar medium having 15% sucrose, 200 ppm boric acid, 200 ppm magne-

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sium sulphate and 200ppm potassium, nitrate and 1% agar. The pH of the medium was adjusted to 6.5.

Just anthesized flowers were collected from plants growing in the experimental garden. Pollen grains were collected in a clean petri plate and dusted on the germinating medium with the help of a fine brush. The grains were inoculated on coverslips having a drop of the basal medium. The pollen dusted coverslips were inverted and placed over metalic rings prefixed to glass slides lined with petroleum jelly. The inoculated slides were incubated at 30°C, the optimum temperature for the species, for 2 hours. Germinating pollens were fixed with a drop of FAA. Five replicate slides were maintained for all treatments. Germination percentage was calculated from 5 microscopic fields chosen randomly per slide and the pollen tube length was measured from at least 50 pollen tubes.

For gamma irradiation, freshly collected flowers were exposed to the radiation (1-800 krad) in a gamma chamber having radioactive source Co^{60}, emitting gamma rays at the rate of 66.95 rad/sec. Percent stimulation/inhibition induced by radiation over control was also calculated using following formula:

$$\underset{(+)\qquad(-)}{\text{Stimulation/inhibition}} = \frac{\text{Treated—control}}{\text{control}} \times 100$$

Result and discussion

Pollen germination and pollen tube growth are influenced by plant growth hormones (Johri *et al.*, 1977). The data of present study dealing with the effects of growth hormones on pollen germination and pollen tube growth of *Solanum marginatum* are given in Table 1. It was found that kinetin concentrations (1-25 ppm) inhibited both pollen germination and pollen tube growth. Compared to 1 and 5 ppm, 10 and 25 ppm of kinetin induced more pronounced inhibition of pollen germination. Kinetin induced inhibition of pollen germination and pollen tube growth are also known in *S. khasianum* and *S. indicum* (Ravindran & Chauhan, 1986). In *S. sisymbrifolium*, although higher concentrations of kinetin (25 ppm) inhibited pollen germination and tube growth and the lower concentrations (1-10 ppm) stimulated pollen germination and tube growth (Kuruvilla *et al.*, in press). Amongst the four alkaloid yielding solanums, inhibition of pollen germination is induced by varying concentrations of kinetin, e.g. 1ppm (*S. marginatum*), 10 ppm (*S. khasianum*) and 25 ppm (*S. indicum* and *S. sisymbrifolium*). Thus, the pollen of the four alkaloid yielding solanums differ in their sensitivity to kinetin concentrations.

Incorporation of IAA in the medium inhibited both germination (1-25 ppm) and tube growth (5-25 ppm) of *S. marginatum* pollen. All the concentrations of IAA which induced inhibition of pollen germination and pollen tube growth were almost equally effective (Table 1). Thus, *S. marginatum* pollen has response similar to *S. khasianum*, *S. indicum* and *S. sisymbrifolium*. Pollens of many species contain auxin in sufficient amount for optimal growth (Linskens & Kroh, 1970). Exogenous application of auxin stimulates pollen tube elongation only if auxin content of the pollen is below threshold level (Brewbaker & Majumder, 1961). Since even low concentrations of auxin induced inhibition of pollen germination and tube growth, it appears that *S. marginatum* pollen has optimal concentration of endogenous IAA and does not require exogenous supply of IAA for its germination and tube growth.

In the present study all the concentrations of GA_3 (1-25 ppm) inhibited germination of *S. marginatum* pollen but pollen tube elongation was insensitive to GA_3 (Table 1). GA_3 had similar effects on germination of *S. khasianum* pollen (Ravindran & Chauhan, 1986). However, *S. marginatum* pollen is more sensitive to GA_3 than *S. khasianum* as inhibition of germination in *S. marginatum* pollen occurred at 1 ppm GA_3 while in case of *S. khasianum* pollen it occurred at 5 ppm. Unlike this, lower concentrations of GA_3 stimulated pollen germination and pollen tube growth in *S. indicum* (Ravindran & Chauhan, 1986) and *S. sisymbrifolium* (Kuruvilla *et al.*, in press). Thus different species of solanums have differential sensitivity to GA_3 concentrations.

The effect of gamma radiation on pollen germination and tube growth of *S. marginatum* is presented in Table 2. 200 krad dose of gamma rays promoted pollen germination while higher doses induced inhibition. Although pollen germination was stimulated at 200 krad pollen tube elongation remained inhibited. *S. marginatum* pollen was insensitive to doses lower than 200 krad so far as germination and tube

Table 1—Effect of growth hormones on pollen germination and pollen tube growth of *S. marginatum*

Treatment	ppm	Pollen germination (%)	Pollen tube length (μm)
Control	0	57.27±1.93	144.45± 9.30
Kinetin	1	46.90±0.29*	86.55±13.65
	5	48.01±0.79*	86.55±10.50
	10	37.56±3.36*	88.50±10.54*
	25	41.22±5.51*	82.05± 6.61*
IAA	1	44.14±5.51*	123.45±12.77
	5	42.36±3.48*	94.05± 8.19*
	10	39.07±2.50*	90.45± 2.79*
	25	35.93±4.23*	73.95±16.46*
GA_3	1	48.15±2.03*	124.50± 9.76
	5	45.26±2.90*	126.55± 1.80
	10	40.35±1.75*	125.55± 2.65
	25	44.21±1.51*	126.45± 1.00
LSD		8.23	25.87

* Significantly different from control at p = 0.05

± S E

Table 2—Effect of gamma radiation on pollen germination and pollen tube growth of *S. marginatum*

Treatment	krad	Pollen germination (%)	Pollen tube length (μm)
	0	79.45±0.97	162.60±0.42
	1	76.75±0.22	147.90±0.50
	50	75.77±3.23	132.50±0.90
	100	81.57±2.75	132.00±1.07
LSD		6.44	34.20
	0	63.50±0.58	299.55±0.83
	200	76.92±0.95 (+21.13)*	259.05±0.67 (—13.52)*
	500	36.36±4.23 (—42.74)*	95.55±0.78 (—68.00)*
	800	0(—100.00)*	0(—100.00)*
LSD		7.60	25.35

* Significantly different from control at p=0.05

± S E

Inhibition (—)/Stimulation (+)

elongation are concerned. LD_{50} values for germination and tube elongation were between 500-800 krad and 200-500 krad, respectively. Thus pollen of *S. marginatum* is highly resistant to gamma radiation. This may be due to the fact that a correlation between pollen grain size and radiosensitivity exists; larger pollen being more sensitive to radiation (Brewbaker & Emery, 1962). The pollen of *S. marginatum* being about 30 μm is small and quite resistant to gamma rays as revealed in this investigation. There is a linear decrease in pollen germination percentage with increasing exposure (Pfahler, 1971). The present study also supports this finding.

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