A COMPARISON OF ETHREL AND GAMMA RAYS EFFECTS ON SHOOT APEX ORGANIZATION AND CELL ENLARGE-MENT IN VEGETATIVE SHOOT APEX OF SOLANUM KHASIANUM CL.

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Abstract

The effects of 'ethrel' (50, 100 and 250 ppm) and acute exposures of gamma rays (2.5, 10.0 and 20.0 kR) on the vegetative shoot apex of *Solanum khasianum* Cl. have been studied. Both ethrel and gamma rays treatments reduced the size of the shoot apex, inhibited meristematic activity and influenced cell elongation in the zone of cell maturation. Whereas ethrel enhanced radial cell expansion; rays promoted axial cell expansion. The opposing effect of radiation and ethylene on cell expansion may be due to the differential effect on the orientation of microfibrils in cell wall. The cytohistological zonation was not disrupted by ethrel treatment. γ -ray exposures, however, induced disorganization of the zonation pattern.

Introduction

Both ethylene and gamma rays affect different phases of plant growth and development. It is suggested that an answer to the growth reaction of the plant should be sought in the responses of the shoot apex (Gunckel & Sparrow, 1961). In the present study an attempt is made to study and compare the effects of ethylene, using ethrel, and gamma rays on the organization and differentiation in the vegetative shoot apex of a medicinal plant—Solanum khasianum Cl. (Choudhury & Rao, 1964).

Material and Methods

Seeds of S. khasianum Cl. (moisture content 14.47%) were given 2.5, 10.0 and 20.0 kR exposures of gamma rays with the help of 60 Co source emitting γ -rays at the rate of 4.1 kR/mn. The seeds meant for ethrel treatment were soaked in different concentrations (50, 100 & 250 ppm) of ethrel for 4 hr at room temperature ($20.0^{\circ}C \pm 1$). The control seeds were soaked in distilled water, instead. All the seeds were rinsed in distilled water just before sowing. The seeds were sown in pots filled with 1:1 garden soil and farmyard manure, covered with fine soil and watered regularly for maintenance of moisture required for seed germination.

The seedlings which germinated on the same day were tagged and collected when these were 5 days old. The collected seedlings were fixed in Randolf's modified Navashin mixture (Johansen, 1940) and stored in 70% alcohol. The shoot apices were dehydrated in T. B. A. series, infiltrated and embedded in paraffin. The apices were microtomed at $8 \mu m$ and stained in Tannic acid-Ferric chloride-Safranin-Fast green combination and mounted in DPX.

For observations only median longitudinal sections of the shoot apices were considered. For treatment atleast five apices were scored and the characters studied included topography of the apex, apex size, tunica, corpus, cytohistological zonation and cell

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expansion. For determining size of the shoot apex height of the apical meristem above youngest leaf primordia and breadth of the meristem (transverse line joining youngest leaf primordia) were taken into consideration. Cell area was calculated with the help of radial and axial dimensions of the cells. For a particular cell area atleast ten cells per apex were scored. The mean values of all the measurement are given in Tables 1-3.

Results

The normal shoot apex

Control apices had a dome shaped topography. Tunica in the control apices was biseriate and made of isodiametric cells having dense cytoplasm. In the cells of both the tunica layers no periclinal divisions were witnessed. The corpus was a small core of randomly divided, non layered cells filled with dense cytoplasm (Fig. 1).

Ethrel treated shoot apices

Compared to the control, the ethrel treated apices were smaller in size (Table 1). The tunica was biseriate and occasionally periclinal divisions were witnessed in the T_2 of apices treated with the higher concentrations of ethrel (100 & 250) ppm). Vacuolation of the tunica cells increased with the increasing concentration of ethrel (Table 2).

Ethrel treated short apices resembled control ones in cytohistological zonation pattern. But the region of cell maturation was much distal to shoot appex summit, compared to control (Table 1). Further, while the radial expansion of the cells was enhanced due to ethrel treatment, the axial expansion was inhibited (Table 1) and this resulted in comparatively small broader cells (Text-fig. 1).



Text-fig. 1--Diagrammatic representation of cell shape in the maturation zone of control and treated apices.

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Irradiated apices

Compared to the control, irradiated apices were smaller in size and the size decreased with increasing exposure (Table 1). The apical organization in the irradiated apices was different from the normal shoot tips. Though the apices given low exposures had

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	Sho	ot Apex			Zone of	Cell Exp	ansion in	the zone o	f cell matu	ration, µm	
Treatment	Topography	Height µm	Breadth $\mu_{\rm m}$	Cytohis- tological zonation	cell ma- turation, distance from	Axial	Radial	Arca	Percentage	injury (—) lation (+)	/Stimu-
					summit µ	e H			Axial	Radial	Area
Control	Dome	105.68	156.59	Present	75.80	23.12	13.16	304.259	Ī	1	
Ethrel				÷						÷	
50 ppm	Dome	103.47	144.97	Present	66.40	18.81	14.94	281.021	-18.64	+13.52	7.637
100 ppm	Dome	79.68	127.82	Present	75.25	18.75	17.15	321.562	—18.90	+30.31	+5.686
250 ppm	Dome-hemispherical	40.84	100.70	Present	80.23	17.31	17.65	305.521	-25.12	+34.11	± 0.414
Gamma rays											
2.5 kR	Dome-hemispherical	52.01	106.79	Present	82.44	23.90	13.16	314.524	+ 3.37	0.00	+3.375
10.0 kR	Hemispherical	34.86	105.13	Ill-orga- nized	59.20	24.90	12.94	322.206	+ 7.69	— 1.67	+5.898
20.0 kR	Flat	36.52	99.6	Absent	50.90	29.05	12.53	363,996	+25.64	- 4.78	+19.669

Table 1—Effect of ethrel and γ rays on the vegetative shoot apex of S. khasianum

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Treatment	Extent	Cell structure	Vacuolation	cell area μ_{m^2}
Control	Biseriate	Isodiametric	Dense cytoplasm.	82,062
Ethrel 50 ppm		"	Little vacuolated	81.780
100 ppm	"	"	"	86.709
250 ppm	"	27	Vacuolated	75.801
Gamma rays 2.5 kR	"	"	Little vacuolated	75.604
10.0 kR	27	"	"	74.601
20.0 kR	Uniseriate	"	Vacuolated	71.309

Table 2—Effect of ethrel and γ rays on the tunica in vegetative shoot apex of S. khasianum

Table 3-Effect of ethrel and γ rays on the corpus in vegetative shoot apex of S. khasianum

Treatment	Cell structure	Vacuolation	Cell area μ m ²
Control	Polyhedral	Dense cytoplasm	78.128
Ethrel			
50 ppm	**	,,	70.133
100 pmm	,,) 7	84.000
250 ppm	22	Little vacuolated	65.590
Gamma rays			
2.5 kR	9 7	"	66.526
10.0 kR	"	Vacuolated	73,292
20.0 kR	"	33	66.436

a biseriate tunica, the shoot tips exposed to 20.0 kR revealed only T_1 (Fig. 3). The T_2 , whenever present, was irregular and broken in these apices. Vacuolation in the tunica cells of the irradiated apices increased with the increasing exposure (Table 2).

Corpus was ill-developed in the irradiated apices (Fig. 3) and the corpus cells showed greater vacuolation in comparison to control (Table 3, Fig. 3).

The cytohistological zonation was ill-organized in the apices exposed to the higher exposures of γ rays. The region of cell maturation, present subjacent to the corpus, was much proximate to the summit in the irradiated apices (Table 1). Compared to the control, cells in the region of cell maturation were bigger (Table 1). However, unlike ethrel, radiation enhanced axial expansion of the cells (Table 1, Text-fig. 1). The radial expansion was inhibited by the γ ray exposures. Disruption of the cells was evident in the apices exposed to 20.0 kR (Fig. 3).

Discussion

Compared to the control, apices of both the treatments (ethrel and gamma-ray treated) were smaller. The decrease in the size increased with the increasing concentration of ethrel and γ ray exposure. While in the ethrel treated apices the topography of the meristem resembled control, in the irradiated apices it varied from dome (2.5 kR) to flat (20.0 kR). Inhibition of shoot growth as a response to ethylene (Wee & Rao, 1979) and γ -irradiation (Cecich & Miksche, 1970) is reported before. Radiation induced flattening of the meristem is attributed to the inhibited meristematic activity (Rai & Singh, 1976).

Apices of the control seedlings had a biseriate tunica and a corpus of a small core of randomly divided non-layered cells just below the tunica. Similar features were evident in the ethrel treated experimental material. However, in the apices of the seedlings raised from irradiated seeds only T_1 was present at the highest exposure. The differential sensitivity of the tunica layers is reported in the literature (Pratt, 1968; Cecich & Miksche, 1970; Chauhan & Singh, 1975). Corpus in γ ray treated apices was ill-developed.

Apices of various ethrel treatments and control exhibited a distinct cytohistological zonation. While apices given low exposures of γ rays revealed an ill-organized zonation pattern, the apices given highest exposure had no zonation. Injury to the zonation was reflected in the vacuolated cytoplasm, poor staining capacity of the meristem and a very distal region of cell maturation. Cells of the eumeristematic region in treated apices were of smaller size, compared to control. All these manifestations are considered to be the indications of inhibited meristematic activity. Ethylene-and radiation-induced inhibition of meristematic activity is attributed to reduced DNA synthesis in the treated material (Apelbaum & Burg, 1972; Burg *et al.*, 1972; Iqbal, 1976). Fan and Maclachlan (1967) has reported a direct association between new DNA synthesis and meristematic activity.

In the region of cell maturation ethrel treatments enhanced radial expansion of the cells and inhibited axial enlargement. Gamma-ray exposure, on the other hand, stimulated axial expansion and inhibited radial enlargement of the cells. Thus, the shape of the cells in the ethrel treated apices differed from that in the irradiated apices. The stimulation and inhibition of both axial and radial cell expansion by ethrel and γ rays were concentration and exposure dependent. According to the multinet hypothesis of cell wall, the direction of cell enlargement is influenced by orientation of the microfibrils in the cell wall (Houwink & Roelofsen, 1954). Thus the opposing effect of radiation and ethylene on cell expansion may be due to the differential effect on the orientation of microfibrils in cell wall.

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Explanation of Plate

1-3. Median longitudinal sections of five days old S. khasianum shoot apices. X 325. 1, Control; 2, 250 ppm ethrel treated; 3, 20.0 kR γ -ray irradiated.



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Chauhan-Plate 1