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Biological Effects of High Background Radioactivity: Studies on Plants Growing in the Monazite-bearing Areas of Kerala Coast & Adjoining Regions

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Areas with naturally occurring high background radioactivity exist along the coastal stretches of Kerala State and adjoining regions in South India. These areas support rich vegetation and offer immense opportunities to study *in situ* the long-term effects of chronic irradiation on plants. Cytological studies indicate that the incidence of meiotic abnormalities is higher in samples from the radioactive areas as compared to those from control belt. Detailed analysis of the data shows that relationships exist between the external radiation level, internal radionuclide content and cytological abnormalities. The magnitude of internal radiation dose received by plants arising out of their radionuclide content has been computed. The total (external + internal) radiation dose received by the plants is correlated with the cytological abnormalities.

THE impressive developments in atomic research in recent years and the increasing application of nuclear energy in many fields of human endeavour have brought to the forefront serious problems concerning the long-term effects of radiation on man and his environment. During recent years several chronic irradiation studies have been carried out both under laboratory and field conditions, using man made radioactive sources, to evaluate biological effects of such radiation exposures.

It has been demonstrated that, in mice, gamma irradiation at low doses and dose rates results in increased mutation rate and reduction in the life span as well as litter size which suggests lowered reproductive capacity of the irradiated populations[1-3]. Mericle and Mericle[4] have reported an increase in the somatic mutation frequency in *Tradescantia*, clone-02, exposed to external radiation from the radioactive minerals of the Colorado dike. Blaylock's[5] studies on the *Chironomus tentanus* population in the radioactive waste disposal area of Oak Ridge National Laboratory have revealed the occurrence of chromosomal aberrations in very low frequency.

While the above investigations have indicated the nature of biological effects of chronic irradiation, there is a distinct lack of quantitative data on the real long-term consequences to biological systems exposed to continuous doses of radiation for very long periods of time. Areas with naturally occurring high background radioactivity offer unique opportunity for obtaining this information.

The coastal stretches of Kerala and Madras States in South India, which are among the best known of the high radiation areas in the world, have background radiation levels as high as 5·0 mR/hr which are up to 250 times greater than the normal background levels of 0·02 mR/hr. The radioactivity in these areas is due to the presence in the soil of monazite which contains principally thorium-232 and its radioactive daughter elements. The monazite belt is not continuous but is interrupted by non-radioactive or nearly non-radioactive areas. All these regions support rich vegetation offering excellent possibilities for studying *in situ* the effects of chronic irradiation in plants.

Preliminary observations from this laboratory on cytological effects in plants growing in the high background radiation areas indicated a higher incidence of cytological aberrations in the irradiated populations as compared to that in the controls. In the course of the survey of the flora of this area, morphologically abesrant plants have been observed among species of *Crotalaria* and *Lochnera* which showed flower and leaf abnormalities. Accumulation of radionuclides of the thorium series in plants growing in these regions has also beer demonstrated and significant positive correlations have been obtained between the radionuclide content of the plants and the soil activity for a large number of species[6,7]. The present paper reports data on cytological abnormalities and pollen sterility in plants growing in the high radiation areas. Relationships between external radiation level, internal radionuclide content and the cytological effects have also been examined.

Materials and Methods

Cytological studies have been carried out for those plants which commonly occur in these coastal areas and for which a large number of samples were easily available. Altogether there were seven such species, viz. *Crotalaria verrucosa* L. ($2n = 16$), *Lochnera rosea* Reichb. ($2n = 16$), *Calotropis gigantea* R. Br. ($2n =$

22), *Launaea pinnatifida* Cass. ($2n = 18$), *Tridax procumbens* L. ($2n = 36$), *Ipomoea pes-caprae* Sweet (*Ipomoea biloba* Forsk.) ($2n = 30$) and *Croton bonplandianum* Baill ($2n = 20$). Flower buds were collected from these plants for meiotic studies. External radiation levels at the site of collection of the samples were recorded with a Geiger-Muller beta-gamma survey meter. To provide a realistic evaluation of the data on plants from the high radiation areas, the study included plants growing in selected areas having normal background radiation levels and ecological conditions similar to the exposed populations. Cytological screening was carried out at different stages of meiosis using the acetocarmine technique. For each plant sample over 500 cells were scored. Incidence of pollen sterility was also recorded. Measurements of the internal radionuclide content in the plant samples were carried out through assay of total alpha activity using low background ZnS(Ag) scintillation counters.

Results and Discussion

Cytological screening of the plant samples from the monazite-bearing high radiation areas revealed various types of meiotic abnormalities. At diakinesis, increased numbers of nucleoli, cytomixis, quadrivalents, interlocked bivalents, haploidy and chromosome fragments were observed (Plate I, Figs. 1-6). The irregularities at anaphase I and II were precocious separation of bivalents, unequal distribution of chromosomes, bridges, fragments, diads and pentads (Plate II, Figs. 7-12). In addition to these, extrusion and non-disjunction of chromosomes were recorded in the exposed as well as control samples; these abnormalities were relatively higher in the former. The various types of abnormalities occurring at different stages of meiosis for each sample were pooled and the percentage was calculated. These values are included in Table 1.

Data on the background radiation levels at the site of collection of the plant samples, the cytological abnormalities and the pollen sterility are given in Table 1. It is apparent from the data presented there that the incidence of cytological abnormalities and pollen sterility was higher in samples from the radioactive areas.

Statistical analysis of the data for the number of species under study showed significant positive

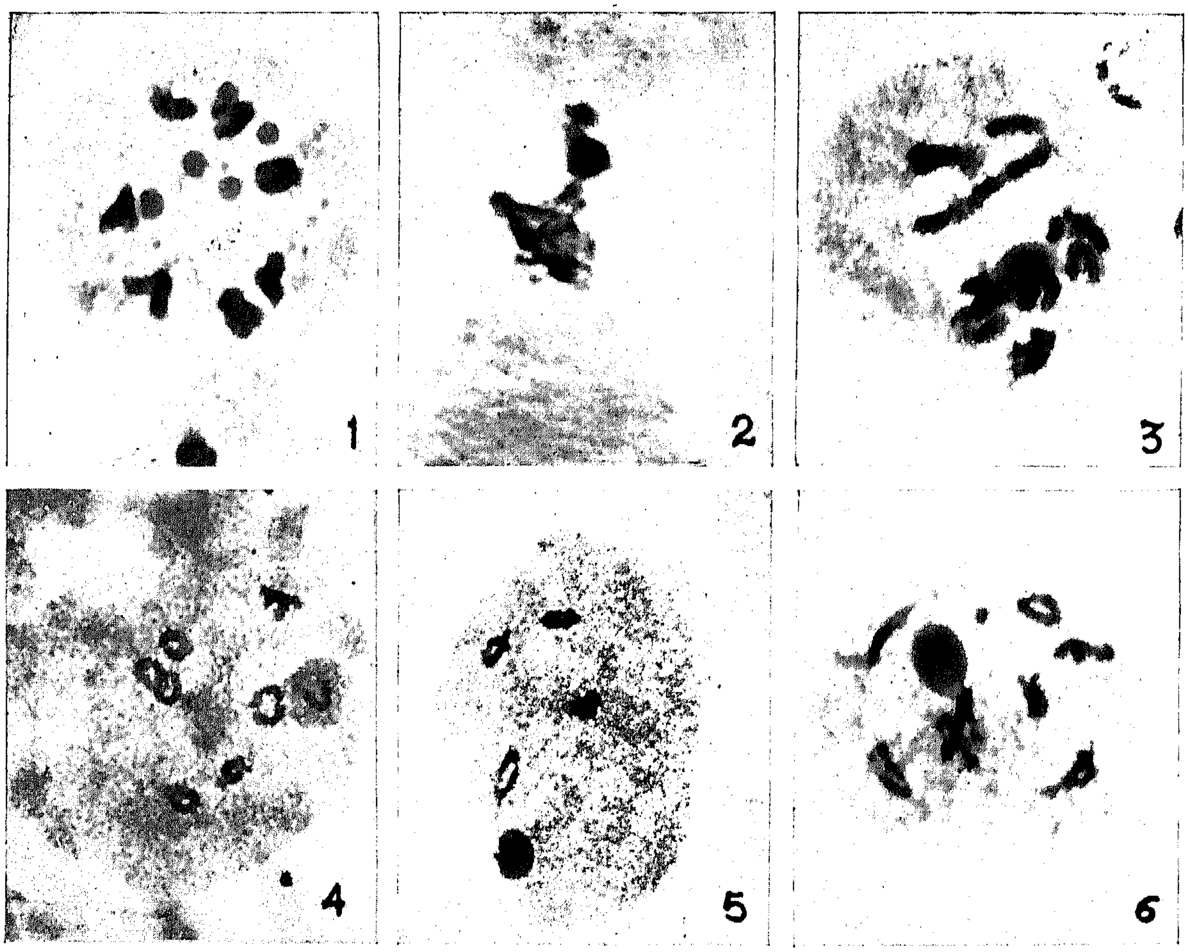

Plate I — Cytological abnormalities at diakinesis × 1250 [Fig. 1: Increased number of nucleoli in *Crotalaria verrucosa*. Fig. 2: Cytomixis in *Lochnera rosea*. Fig. 3: Quadrivalent in *Launaea pinnatifida*. Fig. 4: Interlocked bivalents in *C. verrucosa*. Fig. 5: Haploid cell in *C. verrucosa*. Fig. 6: One fragment in *C. verrucosa*] correlation between the external radiation level and the observed cytological damage (Table 2). Correlations could not be worked out for *Calotropis gigantea* and *Croton bonplandianum* since cytological damage in these species was insignificant.

The radiosensitivity of plants varies from species to species as shown by the differences between the slopes of the regression of cytological abnormalities on the background levels (Table 2). The sensitivity of different plant species to high background radiation showed positive linear correlation with their interphase chromosome volume (ICV) (Nayar, G. G., George, K. P. & Gopal-Ayengar, A. R., unpublished data).

For the plant population under study significant positive correlations have been obtained between the external radiation level and the internal radionuclide content[7]. Since data were available on total alpha activity in the plant body and cytological abnormalities for a number of samples of *Lochnera rosea*, correlations were attempted between these parameters. Correlation coefficients and the regression equations which indicate significant positive correlation are given in Table 2.

While evaluating the regressions it should be realized that variables such as the age of the plant at the time of collection, fluctuations in the external radiation levels to which plants are exposed throughout their life-cycle and the variable amount of energy released within the plant body as a result of variations in the internal radionuclide content might be responsible for the observed scatter of the experimental data.

Radiobiological considerations of internal radiation exposure due to the absorbed radionuclides — The gamma ray spectra of plants[6,7] as well as available evidence from controlled nutrient culture experiments on the uptake of radionuclides of the natural uranium and thorium series[8-10] indicated that the radioactivity of the plant samples was, in the main, due to entry of the long-lived isotope of radium, ^{228}Ra, in the plant and its subsequent radioactive decay resulting in the growth of alpha, beta and gamma emitting daughter radionuclides. It was possible, therefore, through Bateman equations[11,12] to compute the levels of ^{228}Ra and its daughters in plants from the total alpha activity data. The radionuclide levels were then utilized to calculate

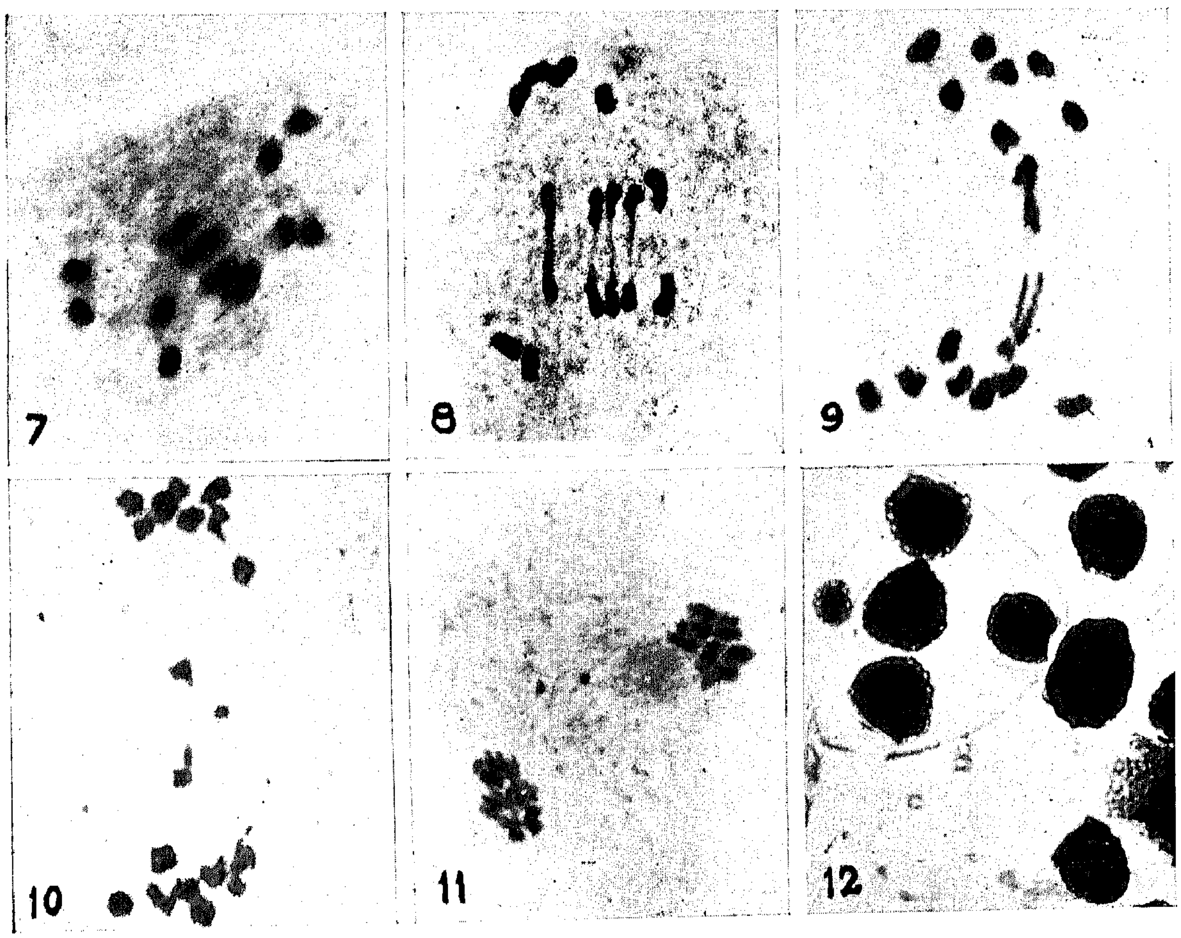

Plate II — Cytological abnormalities at anaphase I and telophase II [Fig. 7: Precocious separation of bivalents in *C. verrucosa* ×1250. Fig. 8: Unequal distribution of chromosomes in *C. verrucosa* ×1250. Fig. 9: Bridges in *C. verrucosa* ×1250. Fig. 10: Bridge and one fragment in *L. pinnatifida* ×1500. Fig. 11: Two fragments in *L. pinnatifida* ×1250. Fig. 12: Pentads in *Tridax procumbens* ×750]

TABLE 1 — CYTOLOGICAL ABNORMALITIES AND POLLEN STERILITY IN DIFFERENT PLANT SPECIES FROM HIGH AND NORMAL BACKGROUND RADIATION AREAS

Place of collection of the sample	Background radiation level (mR/hr)	Cells showing cytological abnormalities (% of total)	Pollen sterility (% of total)
	Lochnera rosea		
Veli	0·02*	0·76	1·39
do	0·02*	1·87	0·72
do	0·02*	1·02	0·70
do	0·02*	0·48	0·99
Quilon	0·02*	0·52	1·00
Chavara	0·7	1·63	0·84
do	0·8	3·10	0·92
do	1·0	2·06	0·89
do	1·1	1·42	2·50
Manavalakurichi	1·2	2·77	2·78
do	1·2	1·81	4·34
	Ipomoea pes-caprae		
Quilon	0·02*	0	0·41
Midalam	0·6	0·57	0·43
Chavara	1·0	0·63	0·59
do	1·7	0·73	0·76
do	2·0	1·47	2·30
Midalam	2·0	1·99	7·88
	Crotalaria verrucosa		
Quilon	0·02*	0·55	1·59
Manavalakurichi	0·6	1·44	n.d.
Midalam	0·7	2·49	9·19
Chavara	1·5	2·40	n.d.
Midalam	0·8	2·01	2·27
	Launaea pinnatifida		
Veli	0·02*	1·07	1·11
Quilon	0·02*	1·08	1·10
Chavara	0·4	3·52	1·26
Manavalakurichi	0·8	3·03	2·45
Midalam	2·0	4·94	4·27
	Tridax procumbens		
Veli	0·02*	1·40	n.d.
Chavara	0·3	3·40	n.d.
Manavalakurichi	0·6	2·90	n.d.
do	1·0	5·20	n.d.
do	1·4	5·10	n.d.
	Calotropis gigantea		
Veli	0·02*	0	n.d.
Chavara	0·20	0	n.d.
Manavalakurichi	0·70	0	n.d.
Neendakara	0·50	0	n.d.
Manavalakurichi	1·00	0·28	n.d.
Chavara	1·30	0	n.d.
do	1·30	0	n.d.
	Croton bonplandianum		
Veli	0·02*	0	n.d.
do	0·02*	0	n.d.
do	0·02*	0	n.d.
Midalam	0·50	0·61	n.d.
do	0·60	0	n.d.
Kadiapatanam	0·80	0	n.d.
Midalam	1·20	0	n.d.
do	1·50	0	n.d.
do	1·60	0	n.d.

n.d. = not determined.
*Control.

the magnitude of internal radiation dose received by the plants.

The integral internal dose delivered by an alpha or beta emitter was computed using the following equation[13]:

$$D_t = 73{\cdot}8 \times E \times C_0 \times T_{eff}(1 - e^{-\lambda_{eff}t}) \times M$$

where D_t = integral internal dose over time t (730 days in the case of *Lochnera rosea*) in rads; E = energy of alpha or beta (av.) particle in MeV; C_0 = radionuclide content at the time of collection of the plant in μC/g tissue; T_{eff} = effective half-life of the radionuclide in plant which is equal to the physical half-life in the absence of an established excretory mechanism in plants for radionuclides of the thorium series; $\lambda_{eff} = 0{\cdot}693/T_{eff}$; t = 730 days for *Lochnera rosea* species; and M = weight of the plant tissue in g.

Since gamma rays are far more penetrating than alpha or beta particles, considerations of the size and shape of the irradiated tissue that do not exist for alpha or beta dosimetry have to be taken into account in calculating the gamma dose. The integral internal dose delivered by a gamma emitter was computed using the following equation[13]:

$$D_t = 0{\cdot}0346 \times C^0 \times T_{eff} \times \Gamma \times \bar{G}_p(1 - e^{-\lambda_{eff}t}) \times f \times M$$

where D_t = integral internal gamma dose over time t (730 days in the case of *Lochnera rosea*) in rads; $\Gamma = 1{\cdot}50 \times 10^5 \times \mu w \times E_\gamma$ in cm²-r/mC-hr; μw = absorption coefficient of gamma radiation in tissue equivalent of water; E_γ = energy of gamma radiation in MeV; $\bar{G}_p$ = average geometrical factor calculated for a cylindrical model of radius 1 cm and variable height as computed from the relation: volume of cylinder (V) = $\pi r^2 h$, taking (V) for cylinder of unit density to be equal to the fresh weight of the plant; f = photofraction of gamma radiation having energy E_γ; C_0, T_{eff}, λ_{eff}, t and M have the usual significance.

In the present paper we have included the data on the estimated integral internal dose for seven plant samples of *Lochnera rosea* for which data on cytological abnormalities were available (Table 3). The magnitude of internal radiation dose computed for sample 3 is rather surprising since this plant sample was collected from a control area. It is felt that the reported value is, in all probability, due to contamination of plant material. Data on the integral external dose received by the plants have also been included in the table.

Data presented in Table 3 indicate that alpha emitting radionuclides are responsible for about 60% of the total internal dose received by the plants. The contribution from gamma emitters is nearly 40% while that of beta emitters is only 0·01%. These observations substantiate our earlier assumption that total alpha activity in plants can be taken as an index of the internal radionuclide content for the purpose of working out correlations with the observed cytological damage.

The correlation coefficients and the regression equations relating the integral internal and external doses and the meiotic abnormalities for *Lochnera rosea* plants are given in Table 4. It is evident that

TABLE 2 — CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS SHOWING THE RELATIONSHIP BETWEEN VARIOUS PARAMETERS

Species	External radiation level versus cytological abnormalities		Internal radionuclide content versus cytological abnormalities		Internal radionuclide content versus external radiation level*	
	Corr. coeff.	Regr. eq.	Corr. coeff.	Regr. eq.	Corr. coeff.	Regr. eq.
Lochnera rosea	0·622†	$y=0{\cdot}97x+0{\cdot}70$	0·783†	$y=9{\cdot}21x-5{\cdot}38$	0·526‡	$y=0{\cdot}88x+0{\cdot}45$
Ipomoea pes-caprae	0·885†	$y=0{\cdot}77x-0{\cdot}04$	—	—	0·813‡	$y=0{\cdot}87x+0{\cdot}18$
Crotalaria verrucosa	0·817†	$y=1{\cdot}27x+0{\cdot}84$	—	—	0·719‡	$y=0{\cdot}76x+0{\cdot}10$
Launaea pinnatifida	0·890†	$y=1{\cdot}83x+1{\cdot}50$	—	—	0·992‡	$y=9{\cdot}27x-1{\cdot}20$
Tridax procumbens	0·882†	$y=2{\cdot}72x+1{\cdot}66$	—	—	—	—
Calotropis gigantea	—	—	—	—	0·602†	$y=2{\cdot}19x+0{\cdot}08$
Croton bonplandianum	—	—	—	—	0·804‡	$y=0{\cdot}71x+0{\cdot}34$

*Data of Mistry *et al.*[7].
†Significant at 5% level.
‡Significant at 1% level.

TABLE 3 — DATA ON INTERNAL AND EXTERNAL RADIATION DOSES RECEIVED BY *Lochnera rosea* PLANTS GROWING IN HIGH AND NORMAL BACKGROUND RADIATION AREAS OF THE KERALA COAST AND THE OBSERVED CYTOLOGICAL ABNORMALITIES

Sl No.	Radiation level at the site of collection (mR/hr)	Integral internal dose (rads)				Integral external dose (rads)	Total (internal + external) dose (rads)	Cells showing cytological abnormalities (% of total)
		Alpha	Beta ($\times10^{-3}$)	Gamma	Total			
1	0·02*	nil	nil	nil	nil	0·33	0·33	0·52
2	0·02*	nil	nil	nil	nil	0·33	0·33	0·76
3	0·02*	0·25	0·43	0·16	0·41	0·33	0·74	0·48
4	0·7	0·25	0·43	0·17	0·42	11·65	12·07	1·63
5	0·8	1·55	2·70	1·01	2·56	13·32	15·88	3·10
6	1·0	0·85	1·48	0·55	1·41	16·64	18·05	2·06
7	1·1	0·56	0·97	0·37	0·93	18·30	19·24	1·42

*Control.

significant linear relationships exist between total (internal + external) dose and the observed cytological damage. Correlations were also worked out for internal and external doses separately and the cytological abnormalities. The correlation coefficients and regression equations are included in Table 4. It is seen that the slope value for the regression of internal dose on cytological damage is higher by an order of magnitude than the corresponding value for the regression of external dose on cytological damage. In the present situation, the radiobiological implications of these differences cannot be clearly evaluated due to the possible interaction between external and internal radiation in the production of the observed cytological damage. However, these findings appear to be compatible with the results of our earlier studies[14] on *Tradescantia*, clone 02, plants grown in monazite containing sand culture which indicated that the contribution of absorbed radionuclides to biological damage, as measured in terms of the frequency of somatic mutation rates of staminal hair, is significantly greater than that of external radiation exposure.

The pattern of localization of radionuclides of the thorium series in plants at the cellular level has not been investigated in detail. However, it is of interest to note that radium which, in all probability, is the radionuclide that enters plants in significant amounts is related chemically to the divalent ion calcium which has been found to be associated with the nucleus[15]. Therefore, it is likely that a fraction of the radium absorbed by plants is ultimately incorporated in the nucleus leading to the emission of strongly ionizing radiations in this region of the cell.

TABLE 4 — CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS SHOWING THE RELATIONSHIPS BETWEEN INTEGRAL INTERNAL AND EXTERNAL RADIATION DOSES AND CYTOLOGICAL ABNORMALITIES FOR *Lochnera rosea* PLANTS

	Corr. coeff.	Regr. eq.
Integral (internal + external) radiation dose versus cytological abnormalities	+0·792*	$y=0{\cdot}0862x+0{\cdot}5974$
Integral internal radiation dose versus cytological abnormalities	+0·934†	$y=0{\cdot}9689x+0{\cdot}6254$
Integral external radiation dose versus cytological abnormalities	+0·749*	$y=0{\cdot}0878x+0{\cdot}6540$

*Significant at 5% level.
†Significant at 1% level.

The data presented here offer direct evidence on the cytological effects of high background radiation and serve to indicate the probable magnitude of damage to plant populations exposed for very long periods of time.

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