[Ra]diation Botany, 1970, Vol. 10, pp. 287 to 292. Pergamon Press. Printed in Great Britain.

ON THE BIOLOGICAL EFFECTS OF HIGH BACKGROUND RADIOACTIVITY: STUDIES ON *TRADESCANTIA* GROWN IN RADIOACTIVE MONAZITE SAND

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(*Received* 3 *June* 1969)

Abstract—*Tradescantia*, clone 02, plants were grown in garden soil and were exposed to low level external radiations ranging from 0·08 mR/hr to 1·3 mR/hr or were raised in radioactive monazite sand cultures. In the experiment on external exposure alone, one group of plants was irradiated from transplanting while another group was irradiated from flowering. The occurrence of somatic mutations in the staminal hairs was recorded from flowering, for a period of over sixty days. After the specified period of growth, the plants from the monazite cultures and control were sacrificed and assayed for the absorbed radionuclides, especially for the alpha emitters. The radionuclide content in the plant body did not show a direct relation with the amount of monazite in the cultures. It appears that the contribution from the absorbed radionuclides is much more important in the production of biological damage than external irradiation alone.

Résumé—Des plantes de *Tradescantia* du clone 02 ont été mises en croissance au jardin et exposées à des basses doses externes de radiations allant de 0,08 mR/hr à 1,3 mR/hr ou bien ont été culturées sur du sable de monazite radioactive.

Dans l'expérience sur l'irradiation externe seule, un groupe de plantes a été irradié à la transplantation alors qu'un autre l'était à la floraison. L'apparition de mutations somatiques des poils staminaux a été observée à la floraison pendant une période de soixante jour. Après la période de croissance spécifiée, les plantes provenant des cultures sur monazite ainsi que le témoin ont été sacrifiés et testés du point de vue des radionudides absorbés spécialement du point de vue de l'émission des particules alpha. Le contenu des plantes en radionuclides ne montre pas de relation directe avec la quantité de monazite des cultures. Il apparaît que la contribution des radionuclides absorbés est plus importante dans l'induction du dommage biologique que l'irradiation externe seule.

Zusammenfassung—*Tradescantia*-Pflanzen (Klon 02) wurden auf Gartenboden gezogen und wurden niederen Aussenbestrahlungen ausgesetzt zwischen 0,08 mR/Std. und 1,3 mR/Std. oder in radioaktiven Monatzitsandkulturen gezogen. Bei dem Experiment, in dem nur Aussenbestrahlung vorgenommen wurde, wurde eine Gruppe der Pflanzen nach dem Überpflanzen bestrahlt, während eine andere nach der Blüte bestrahlt wurde. Es waren somatische Mutationen der Staubblatthaare nach der Blüte für eine Zeit von über 60 Tagen zu verzeichnen. Nach der angegebenen Wachstumsperiode wurden die Pflanzen der Monatzitkulturen und der Kontrollen geerntet und auf die aufgenommenen Radionukleotide hin untersucht, besonders auf α-Strahler. Der Radionukleotid-Gehalt im Pflanzenkörper zeigte keine direkte Beziehung zu dem Monatzitgehalt in den Kulturen. Es scheint, dass der Beitrag der absorbierten Radionukleotide viel bedeutender ist für die Bildung biologischer Schäden als Aussenbestrahlung allein.

INTRODUCTION

THE MAGNITUDE of biological damage caused by high background radiations has been of great concern to biologists and the significance of such studies has been stressed earlier.[8,18] The biological damage from these natural radiations is an overall effect of radiations, both from external and from the absorbed radionuclides. Since the background radiation levels are very low and the period of exposure required to have an observable biological damage is very long, the experimental approach to this problem remains difficult.

The data now available on populations exposed to high background radiations are inconsistent within themselves. A high incidence of chromosomal damage in plants growing in the monazite bearing high radiation areas of Kerala and Madras States has been reported earlier.[4] Studies on the rat populations of these areas did not reveal any evidence of genetic damage as against the control populations.[10] Sparrow and Singleton have reported a significant increase in micronuclei when *Tradescantia paludosa* plants were exposed to 0·41 R/day.[16] Increased incidence of chromosomal damage has been observed in insect populations from the radioactive waste disposal areas of Oak Ridge National Laboratory.[2] Mericle and Mericle have reported a high frequency of somatic mutations in *Tradescantia*, clone 02, exposed to high background radiations from Colorado dike areas.[11,12] A significant increase in the incidence of somatic mutation has been recorded by Sparrow *et al.* in *Lilium testaceum* exposed to 0·170 R/day.[17] However, BAILEY and LUQUIRE[1] could not find any chromosomal variations among *Tradescantia* plants growing in the Alabama region as compared to those growing in non-radioactive areas. To gather more specific information on the effects of low level radiations on the biological systems, the present study was undertaken.

MATERIALS AND METHODS

Tradescantia, clone 02, a hybrid for flower colour (obtained from Dr. A. H. Sparrow, Brookhaven National Laboratory, USA) has been taken as the experimental material. The tissue selected for the present study is the staminal hairs. The suitability of this material for radiobiological studies as well as the mode of development of the staminal hairs on the filament has been reported earlier.[3,15] An epidermal cell in the staminal filament divides in such a way that one of the daughter cells protrudes obliquely out. This cell is meristematic and becomes the terminal cell of the hair, continuing division until the fully grown hair is formed. A subterminal cell in the hair usually divides once after its formation. Very rarely divisions of the interstitial cell also occur. Each staminal filament bears about 40–60 such hairs, each hair being a chain or filament of cells. Thus the hair is almost entirely developed from a single terminal cell.

To simulate the conditions existing in nature, plants were grown in monazite and sand mixtures of various proportions. Monazite is radioactive due to the presence of ^{232}Th and its radioactive daughter elements. Since ^{232}Th has a half life of $1{\cdot}39 \times 10^{10}$ years, the activity remains constant for a very long period. Thus, we have an ideal system in which plants are being grown under conditions similar to those existing in the radioactive areas. The experimental set up was such that plants were grown either in garden soil and were exposed to low level external radiations or were raised in monazite and sand cultures. The radiation levels were recorded by using a G–M counter.

Experiment 1

Plants exposed to external radiation—to have the same quality of radiations present in the radioactive monazite areas, a column of monazite was used as the radiation source. *Tradescantia*, clone 02, plants raised in pots were kept at various distances from the source to receive external dose rates ranging from 0·08 mR/hr to 1·3 mR/hr. The experiment was arranged in such a way that one group of plants was irradiated continuously from the time of transplanting while another group was irradiated from flowering.

Experiment 2

Growing plants in monazite and sand cultures:—Subterranean root stocks of *Tradescantia*, clone 02, were raised in cultures of monazite and sand mixtures in pots under field conditions. The details of the experimental set up are given in Table 1.

Table 1. *Details of the experiment where* Tradescantia *plants were grown in monazite sand mixtures*

Sample no.	Monazite: sand (Ratio)	Radioactivity, mR/hr		
		Root zone	On surface	1 ft above
1	0:1 (Control)	0·03	0·03	0·03
2	1:3	9·0	4·5	1·4
3	1:1	14·0	8·0	1·8
4	3:1	20·0	11·0	2·6

The ratio of monazite to sand was varied; sand alone to 3:1 (monazite: sand). The highest exposure rates obtained in the monazite sand cultures were 2·60, 11·00 and 20·00 mR/hr, one foot above, on the surface and in the sand respectively. The control value at all levels was 0·03 mR/hr.

To all cultures about 25 cc of Hoagland's nutrient solution was added once a week in addition to normal watering. Data on somatic mutations in the staminal hairs were recorded from first flowering, selecting flowers at random. The scoring of mutation was done for a period of over two months taking data on alternate days. While scoring a maximum of three flowers was considered from each treatment and control. At the end of scoring, the plants were sacrificed, thoroughly washed, dried, ashed and assayed for the absorbed radionuclides. Whole plants were used for determining the radionuclide content following the methods reported earlier.[14] For the present study only the total alpha activity in the plant body has been estimated since our earlier calculations have indicated that above 60 per cent of the total energy released in the plant body is contributed by the alpha emitters.[6] In the remaining part of this paper the control and the various monazite sand cultures will be referred to as sample 1, 2, 3 and 4 as given in Table 1.

Pink cells were recorded as mutant cells against the normal blue cells in the staminal hairs. Single cells or group of mutant cells separated by one or more normal cells were considered to have resulted from two separate mutant events. Thus one mutant event can result in either a single mutant cell or a row of mutant cells. The data on somatic mutations for the whole period were pooled.

OBSERVATIONS

Tradescantia, clone 02, plants flowered about eight months after transplanting under the field conditions. The mutant cells appeared as single or multiples in the staminal hairs, terminal or interstitial in position.

Experiment 1

Data on somatic mutations were recorded separately for plants exposed to irradiation from transplanting and for those kept from flowering. The results are represented in Table 2.

It can be noticed from the Table that there is no marked difference in the percentage of stamens with mutation frequency between the irradiated and control samples.

Experiment 2

The plant growth was normal in all cultures except in sample 4 where the plants showed slight growth inhibition. The percentage of stamens with somatic mutations and the estimated radionuclide content in the plant body are given in Table 3.

It will be observed from Table 3 that the percentage of stamens with somatic mutation frequency was higher in all the treatments than the control and the differences were found to be significant. The maximum difference was noticed in sample 3 followed by samples 4 and 2.

Table 2. Showing the exposure rate, total exposure and the percentage of stamens with somatic mutations in Tradescantia, *clone* 02

External radiation		Somatic mutations in the stamens	
Exposure rate, mR/hr	Total exposure, R	No. of stamens scored	Percentage of stamens with mutant events ±S.E.
Plants exposed from transplanting (280 days)			
0·03 (control)	0·201	642	2·77±0·706
0·08	0·546	264	2·65±1·030
0·16	1·092	300	2·33±0·871
0·32	2·184	276	2·22±0·897
0·65	4·368	558	3·96±0·868
1·30	8·736	546	3·63±0·819
Plants exposed from flowering (42 days)			
0·03 (control)	0·030	306	2·28±0·850
0·08	0·079	210	3·33±0·390
0·16	0·159	288	3·81±1·130
0·32	0·318	288	2·43±0·910
0·65	0·655	288	3·12±1·020
1·30	1·310	300	1·33±0·660

Table 3. Data on exposure, the somatic mutation frequency and the radionuclide content in Tradescantia, *clone* 02, *raised in monazite-sand cultures* (283 *days*)

Sample no.	Total exposure, R			No. of stamens scored	Somatic mutations in the stamens	Radionuclide content μμc/gm of tissue
	Root zone	On surface	1 ft above		Percentage of stamens with mutant events±S.E.	Total alpha emitters
1	0·203	0·203	0·203	414	1·60 ±0·617	Nil
2	61·128	30·564	9·508	330	4·54* ±1·14	8·34
3	95·088	54·336	12·225	168	12·50† ±2·55	11·36
4	135·840	74·712	17·659	132	6·00* ±2·07	9·50

*Significant at 5 per cent level.
†Significant at 1 per cent level.

It will also be seen from the Table that the highest concentration of radionuclides was in sample 3 followed by samples 4 and 2.

DISCUSSION

Earlier studies on a plant population growing in the monazite bearing high radiation areas showed a high incidence of chromosomal damage as compared to the control population and this higher chromosomal damage was found to be correlated with the external radiation level to which the plant species were exposed as well as to the absorbed radionuclide content.(5) Species variation in the radionuclide content has also been reported in plants growing in the monazite areas.(9,14) Since these studies were on a wild population, the total dose to which the plants were exposed could not be evaluated. The background radiation levels in the radioactive belt depend very much on seasonal fluctuations such as rains, wind velocity, tidal flow and other environmental conditions. However, in the present experiments the period of exposure and the total dose received by the plants are known. In the experiment where *Tradescantia*, clone 02, plants were subjected to external radiation alone, no appreciable difference was noticed in mutation frequency between the treatments and the controls even though the exposure rates were several fold higher than the normal background radiation levels. This was true when plants received radiation from young stages or were exposed from flowering. Mericle and Mericle[12] have reported significantly higher mutation frequency in petals of *Tradescantia*, clone 02, exposed to high background radiations (0·25 mR/hr) for a short period at the Colorado dike area containing uranium ore as compared to control plants grown in normal background radiation level (0·05 mR/hr). Somatic mutations even in samples exposed to 1·3 mR/hr from monazite sand having natural thorium ore was not found to be markedly different from that of the control (0·03 mR/hr) even though the period of exposure was much longer. This could be due to the differences in the quality of the radiations emitted by these ores.

When plants were raised in monazite (Experiment 2) they were subjected to both external and internal radiations, the latter from absorbed radionuclides. Under the experimental set up all the plants had normal growth except those raised in culture sample 4 where the plants were less vigorous. This could be due to the high mineral content in the soil leading to toxicity, poor aeration of monazite sand and other physiological factors. Similarly, it could also arise as a result of the reduced activity of the roots of plants growing in the culture.

The higher incidence of somatic mutations noticed in the treated samples was not found to be correlated with the amount of monazite in the culture. But it showed some correlation with the radionuclide content (total alpha emitters) in the plant body. It is also worth noting that there is no direct relation between the amount of monazite in the culture and the absorbed radionuclides. This may be true in lower concentrations of monazite as has been observed earlier.[5,14] It is also probable that there might be some optimal level of monazite in the sand beyond which it acts as a physiological barrier to plants for absorption and normal development. This may be the reason for a higher concentration of radionuclides in sample 3 even though sample 4 had the highest concentration of monazite. Considering the external radiation alone, a higher incidence of mutations should have been expected in sample 4. Slight variation was noticed in the values of spontaneous mutation rates in experiments 1 and 2. This is probably because the experiments were not conducted simultaneously. Environmental variations have been found to alter spontaneous mutation rates in *Tradescantia*, clone 02, to a certain extent.[11,12] Our studies have indicated that the frequency of spontaneous colour variation (yellow to red sectors) in *Canna* var. "Striped queen" varies with seasonal changes. The highest values were recorded during January–April and lowest values June–September period of the year.[7]

The nature of occurrence of single and multiple mutant cells in the staminal hairs of *Tradescantia*, clone 02, has been explained on a chromosomal basis by Nayar and Sparrow.[15] Mericle and Mericle have postulated a modulator system for the sectoring of mutant characters in the same material.[13] The present data if interpreted on a chromosomal basis, fit in well with our earlier observations on plants growing in the monazite bearing high radiation areas.

Since the data on external radiation alone did not reveal any higher incidence of somatic mutations in the staminal hairs and significant increase in mutation frequency was noticed in samples raised in monazite cultures as compared to the control, it is evident that the radiations from the absorbed radionuclides is much more important than external radiations in the production of biological damage. While evaluating the data presented in this paper it should be borne in mind that *Tradescantia*, clone 02, plants were raised under a set up where the amount of monazite was several times higher than what is found in natural conditions in the radioactive belt where the concentration of monazite is about 1 per cent. The interpretation of all the data in terms of the biological damage caused by external and internal radiations could not be evaluated separately due to the possible interaction of these factors in the production of biological damage. However, from the information gathered following the present studies it is quite evident that high background radiations as observed at the monazite areas are capable of causing a detectable amount of genetic damage.

Acknowledgement—We are very grateful to Dr. K. B. MISTRY for assaying the radionuclide content in the experimental samples.

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