Paper 3.

Influence of genome on radiosensitivity.
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The literature on radiosensitivity of plant species has accumulated to a tremendous extent in recent years (Sparrow, 1958; Sparrow and co-workers, 1958, 1960, 1962 and 1963; Swaminathan, 1957; Swaminathan and Natarajan, 1957 etc.). The different factors that affect radiosensitivity have been worked out which have provided a considerable understanding of the mechanism of chromosome breakage by X-rays (Allsopp, 1948; Koller, 1954; Nilan, 1956; Sax and Swanson, 1941 and Sparrow l.c.). Simultaneous to such studies researches were extended to other types of rays specially beta-rays emitted from radioactive phosphorous ($^{32}$P) and gamma-rays from cobalt source ($^{60}$Co) and the data in this direction is not meagre (Armason, Cumming and Spinks, 1948; Shestakov and others, 1955; Sparrow and Singleton, 1953; Sparrow, 1955 and his associates, 1958).

All these researches no doubt had provided considerable clues with regard to the intra and extra cellular factors necessary for controlling radiosensitivity. Some amount of work had also been done on the effect of genomes on radiosensitivity (Bishop, 1952; Conger and Johnston, 1956; Sparrow and his associates, 1958, 1960, 1962a and b and 1963; Swaminathan, l.c.; Swaminathan and Natarajan, l.c.). The genomic influence is generally measured by using diploid and polyploid individuals, but no intensive systematic attempt has yet been given to measure the radiosensitivity in diploids and tetraploids - both artificial and natural. In the present investigation with radioactive $^{32}$P and $^{35}$S therefore an attempt has been made to throw some light in this direction.

While using autotetraploid tissue in the present study roots of seeds
and bulbs treated with colchicine (0.5%) were used. A tetraploid, if allowed to mature, after being raised is supposed to gradually undergo certain intracellular changes which may have a profound influence on radiosensitivity. In order to eliminate such changes tetraploid tissue, as soon as it is produced after colchicine application, was subjected to treatment for comparison with its diploid counterpart. For natural diploid and tetraploid materials Solanum nigrum was used. In addition to this aspect, the plants Lens esculenta, Lathyrus sativus and L. odoratus, all being diploid and with nearly the same chromatin content, were included in this study to get an idea of the response in seed and seedling treatments.
MATERIALS AND METHODS

Materials:

In the present investigation for the study of the relationship between genomes and radiosensitivity in seeds, seedlings and bulbs, the following species were selected:

<table>
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<th>Species</th>
<th>Chromosome number</th>
<th>Parts irradiated</th>
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<tr>
<td>Lens esculenta Moench.</td>
<td>2n = 14</td>
<td>Seeds and seedlings with healthy root tips.</td>
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<tr>
<td>Lathyrus sativus Linn.</td>
<td>2n = 14</td>
<td></td>
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<tr>
<td>Lathyrus odoratus Linn.</td>
<td>2n = 14</td>
<td></td>
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<tr>
<td>Impatiens balsamina Linn.</td>
<td>2n = 14</td>
<td></td>
</tr>
<tr>
<td>Allium cepa Linn.</td>
<td>2n = 16</td>
<td>Bulbs with healthy roots.</td>
</tr>
<tr>
<td>Solanum nigrum Linn.</td>
<td>2n = 24, 48</td>
<td>Seedlings with healthy roots.</td>
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Three sets of investigations were carried out for the study of the radiosensitivity of plant genotypes.

I. In one set of investigations Lathyrus sativus, L. odoratus and Lens esculentus were selected. Seeds and seedlings of these materials with healthy and young roots were subjected to beta (β) irradiation from the source of radioactive phosphorous (Phosphorous - 32) and radioactive sulphur (Sulphur - 35). Radioactive phosphorous (P^{32}) and Sulphur (S^{35}) were applied as inorganic phosphate and sulphate solutions respectively. Seeds and seedlings were kept in
radioactive solutions with strengths 50 \mu c /ml, 100 \mu c /ml and 150 \mu c /ml per 10 seeds or per seedling with an average of 10 young and healthy roots.

II. In the second set of experiments seeds of Impatiens balsamina bulbs of Allium cepa were artificially polyploidised (tetraploid cells in most of the cell population) with 0.5% aq. colchicine solution. The diploid and polyploid materials were then subjected to beta (\beta) irradiation from radioactive phosphorous (P^{32}) and radioactive sulphur (S^{35}) sources. Polyploidised seedlings of Impatiens balsamina and roots of Allium cepa with an average of 10 roots were treated with radioactive solutions having 50 \mu c /ml, 100 \mu c /ml and 150 \mu c /ml strengths.

III. In the last set of experiments naturally occurring diploid (2n = 24) and tetraploid (2n = 48) forms of Solanum nigrum Linn. were irradiated with 20 \mu c /ml, 50 \mu c /ml and 100 \mu c /ml strengths of radioactive phosphorous (P^{32}) and radioactive sulphur (S^{35}) sources. Seedlings of S. nigrum Linn. with an average of 5 to 10 healthy roots were subjected to irradiation.

Seeds of Lathyrus, Lens and Impatiens were collected from local nurseries. Allium cepa bulbs were purchased from local markets and Solanum nigrum seedlings were collected from the attached gardens of the Department of Botany, University of Calcutta.

Methods:

The seeds were soaked in water at room temperature (28°-30°C) for 12 hours and then allowed to germinate in earthenware pots in a mixture of sand, earth and sawdust. The bulbs were also germinated in earthenware pots with sawdust and sand mixture. When the seeds and bulbs germinated and the roots
were about 0.5 cm. to 1 cm. in length, the seedlings and bulbs were uprooted, thoroughly washed in running water and then were subjected to irradiation at a particular dosage. In the case of seed irradiation, seeds were soaked in water at room temperature for 2 to 4 hours before irradiation. In Allium cepa and Impatiens balsamina roots of bulbs and seeds were immersed for 2 1/2 hours and 12 hours in 0.5% colchicine solution respectively so that the majority of the cells in the population became tetraploid. These artificially polyploidised materials were subjected to beta-radiation along with their diploid bulbs with roots and seeds (soaked in water). Four different doses of irradiation were given with each radioactive isotope such as, 20 µC /ml, 50 µC /ml, 100 µC /ml and 150 µC /ml per 10 roots or per 10 seeds.

After irradiation seeds, seedlings and bulbs were washed thoroughly in running water for 2 hours to 3 hours. Seeds were then placed in earthenware pots with sand and earth mixture for germination and bulbs and seedlings were kept in Knop's solution for recovery. Observations were made immediately after irradiation as well as from recovery sets at intervals of 24 hours.

Observations were made in a Zeiss microscope in high power at a magnification of X 900 approximately and photomicrographs were taken when necessary and enlarged suitably. Data were plotted in tabular forms and graphs drawn.
OBSERVATIONS

I. Cytological Characteristics:

Normal karyotypes of three species of pulses (namely, Lathyrus sativus, L. odoratus and Lens esculenta), Allium cepa, Impatiens balsamina and Solanum nigrum were observed before subjecting them to treatment. The frequency of division in non-irradiated bulbs and seeds has also been determined for comparison with the radiated ones.

Lathyrus sativus, L. odoratus and Lens esculenta contain the same mitotic chromosome number $2n = 14$. The chromosomes are in general of medium size having median to submedian primary constrictions. Of the three species chromosomes of Lens esculenta are slightly shorter.

Chromosomes of Impatiens balsamina are also of medium size having $2n = 14$ in the root tip cells. Those of Allium cepa are long and the number is $2n = 16$ in the root tip cells. Primary constrictions are median to submedian in position in both the species.

Chromosomes of Solanum nigrum are of medium size with median to submedian primary constrictions. The chromosome number in diploid plants is $2n = 24$ and in tetraploid plants $2n = 48$ in the root tip cells.

II. Radiation Effects:

For taking data, a number of readings were taken. In each reading the total number of cells in microscope field of observation was counted. The frequency of abnormal cells and abnormalities were counted in relation to the total number of dividing cells. The principal abnormalities are fragments, together with somatic bridges, stickiness and micronuclei with varying numbers.
in resting stages. The number of fragments per cell ranges from one to many. They are of both centric and acentric types, the latter being more frequent. Translocated chromosomes are met with in some cases. The percentage of fragments per cell in relation to its chromosome number was determined.

To radiation damage *Lena esculenta* is less sensitive. In all the cases, the divisional frequency is found to be unaffected. The observed abnormalities are found to be directly proportional to the dose applied.

*Allium cepa* and *Impatiens balsamina* are artificially polyploidised before irradiation. Both diploid and tetraploid forms of *Solanum nigrum* are met with in nature. These three materials respond more or less in the same way. Fragmentation count per cell in tetraploid forms is more than that in diploid forms. But when the percentage count was made in terms of total chromosome number present within the particular cell the percentage of fragments per cell is lower in tetraploid forms than that of the diploid forms. Like the previous experiments here also the sensitivity of chromosomes to radiation varies directly with dosage of strength of the radioactive solution applied. Both diploid and polyploid forms behave in a similar way. Though the percentage of fragments per cell at a particular dosage is higher in tetraploid than the diploid, the sensitivity of tetraploid forms is less than that of its diploid members. Thus the tetraploid forms (both natural and artificially produced) are more radioresistant.

In all the recovery sets of experiments cessation of division was noticed in 24 hours and 48 hours of recovery. Division was observed to occur in 72 hours recovery sets.
DISCUSSION

The data on the effect of radioactive phosphorus (P³²) and radioactive sulphur (S³⁵) on diploid and polyploid tissues of Allium cepa and Impatiens balsamina has yielded rather consistent results. In both cases, except in occasional overlapping of graphs, which is expected due to the variable factors operating, polyploids in general have a higher resistance to their effect as compared to the diploids (vide tables).

In observations carried out just after two hours of treatment in the radioactive isotope, the percentage of fragments shows a remarkable decrease in polyploids as compared to the diploids. This is true for all the concentrations of treatment. Evidently the polyploids are more resistant than the diploids, so far as chromosome breakage is concerned.

This initial difference in the graphs of diploid and polyploid types shows a reversal after recovery for seventytwo hours. In most of the cases, excepting occasionally showing overlapping graphs, polyploids show an increase in the frequency of cells containing fragments. This is true for both Allium cepa and Impatiens balsamina. This discrepancy or rather reversal of the graph can be attributed to the differential capacity of cells containing fragments to survive in diploids and polyploids. In the graphs it appears that polyploids are more resistant to irradiation. Fragmentation percentage i.e., cells with fragments have been found to be lower in them. The same degree of resistance may allow the affected cells to retain their capacity for division, even though some of the chromosomes might have undergone breakage. On the other hand,
affected diploid cells may ultimately lose the capacity of undergoing further division due to chromosome breakage exerting an injurious effect on the cell. In other words the toxicity or injury caused by isotopes can be withstood by polyploids for a longer period in comparison with diploids.

Another remarkable feature is the percentage of fragments per cell in diploids and polyploids. In both Allium cepa and Impatiens balsamina the number of fragments per cell is higher in polyploids though the percentage of fragments which is calculated in terms of chromosome number has been found to be lower in them. Both these features are indications of stronger resistance of polyploids. Though the number of fragments per cell is higher because of the increased chromosome number presenting more surface for attack, yet their increase is double as compared to diploids. On the other hand it is much less than the increase in chromosome surface should allow. This is evidently due to the higher resistance of polyploids than that of their diploid counterparts. This is further reflected in the decrease in the percentage of fragments which is calculated on the basis of the number of fragments in relation to chromosome number.

The results of natural diploids and tetraploids of Solanum nigrum yield more or less comparable data with the ones gathered with diploids and artificially produced polyploids. A similar type of graphs has been obtained specially in recovery sets. But in the initial phase of treatment overlapping of curves has been noted (vide graph No. 3). This overlapping with occasional rise of curve in tetraploids may be due to the fact that in a natural tetraploid several intracellular changes come into operation in the course of evolutionary changes in species. Moreover cryptic structural hybridity might also have been operative in differentiation of S. nigrum tetraploids. As these factors may influence radiosensitivity it is not unlikely that the graphs may often overlap.
in experiments involving so-called natural tetraploids.

With regard to the three species *Lathyrus odoratus*, *L. sativus* and *Lens esculenta*, all of which are diploids with nearly the same chromatin content, the effects have been found to be slightly different from each other (vide graph Nos. 32), both in direct treatment as well as in recovery.

An interesting curve has been obtained in relation to percentage of fragments per cell counted in relation to chromosome number, which is identical in all of them. Both the species of *Lathyrus* show a heavy frequency of fragments in percentage per cell as compared with *Lens esculenta*. This is true both for $p^{32}$ and $s^{35}$ treatments. Evidently this difference can only be attributed to the difference in characters of the genera responsible for the radiosensitivity.
SUMMARY

To study the effect of beta radiation on different genotypes three sets of experiments were designed. For one set of experiments seeds and seedlings of *Lathyrus sativus*, *L. odoratus* and *Lens esculenta* were selected. They contain fourteen chromosomes in their somatic tissue and more or less uniform chromatin content. In another set diploid and artificially polyploids (colchiploids) were irradiated. For this experiment roots of *Allium cepa* (2n = 16) bulbs and seeds of *Impatiens balsamina* (2n = 14) were selected. In the third set of experiments naturally occurring diploid and polyploid types (2n = 24 and 2n = 48 respectively) of *Solanum nigrum* were irradiated. The sources used are radioactive *P*^{32} and *S*^{35} with strengths varying from 50 μc to 150 μc.

The data show that polyploid types, whether naturally occurring or artificially produced, are more radio resistance than the diploids. However in the recovery experiments graphs show a reversal after 72 hours recovery. No division occurs in 24 hours and 48 hours recovery. This discrepancy of the graph can be attributed to the differential capability of cells containing fragments to survive in diploids and polyploids. The radioresistance of polyploids indicates the role of chromatin content in radiosensitivity.

With regard to three leguminous materials the effects have been found to be slightly different. Two *Lathyrus* species were found to be more radiosensitive than *Lens esculenta*, possibly due to the inherent genic differences of the two genera.
REFERENCES


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*Not consulted in original.*