The Mitotic Consequences of Radiation Induced Chromosome Breaks in *Lathyrus sativus* and *Vicia ervilia*¹

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Introduction

The chromosomal aberrations produced after irradiation with ionizing radiations involve breakage of the chromosomes and later reunions. The unions may occur in the original order or a new order following recombination (Stadler 1931, Sax 1940 and Catcheside 1948). The chromosomal aberrations have been stated to be basically of three types. The chromosome types involve breakage of both the chromatids of a chromosome at the same locus, the chromatid types involve breakage of only one of the chromatids (Sax 1940 and Catcheside 1948), and the third type, termed as subchromatid exchanges, results from exchanges between chromosome units smaller than single chromatids (Evans 1962). Irradiation of the interphase cells, however, results in the production of the former types only (Riley 1936, Mather 1938 and Sax 1938).

Sax (1940) was of the opinion that the induction of chromatid breaks occurs if irradiated at prophase when they have already produced two threads whereas irradiation at the resting stage produces chromosome breaks. At the resting stage, the chromosomes were reported (Sax 1957) to be responding as single strands to irradiation. Each of these two types of breaks was again classified to a further degree into single breaks and double breaks, involving two different chromosomes or two loci of the same chromosome, respectively, and the resulting chromosomal abnormalities detectable during mitotic divisions were described in detail by Sax (1940).

The purpose of the present work was to detect and identify the chromosomal aberrations in the different mitotic stages in the root tip cells of *L. sativus* and *V. ervilia* following seed irradiation and to explain the probable causes and effects which resulted into such abnormalities.

Materials and methods

Mature and dry seeds (6-7% moisture) of *Lathyrus sativus* and *Vicia ervilia* were exposed to gamma radiation doses of 5, 10, 15, 20, 25, 30 and 50 Krads from the Co⁶⁰ source of the Queen Mary College (University of London) delivering 5000r

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per minute. The seeds were soaked just after irradiation and kept in water for six hours and germinated on moist filter papers in petri dishes. Soaking and germination were done at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in a thermostatically controlled greenhouse.

Root-tips from the unirradiated controls and irradiated samples of 0.50 to 0.75 cm length were fixed in acetic alcohol (1:3) after 36 to 48 hours from soaking as seeds following higher doses needed longer time to reach the same size. Fixing was done at room temperature (about 20°C). The root-tips were stained with Feulgen and 5 tips of about 2 mm. length were squashed in each slide in a drop of acetocarmine solution. Photomicrographs were taken from preparations made permanent by using euperal essence and euperal.

Results

There are 14 chromosomes in each of the two species (Figs. 1 and 10). There was no chromosomal aberration in the root-tip cells of *L. sativus* and *V. ervilia* control samples. In the irradiated samples, the following types of aberrations were observed (Figs. 2 to 9 and Figs. 11 to 17).

a) Bridges: Single, double, triple (Fig. 14) and multiple bridges, X-shaped (Figs. 3 and 12) and interlocked bridges (Fig. 4). Double bridges were most frequently observed (Fig. 13). The double bridges lasted very long, in some cases upto late telophase, being broken only after the formation of walls in the daughter cells.

b) Fragments and laggards in anaphase (Figs. 5 and 6). Both centric and acentric fragments were present. The fragments were of different lengths and the majority of them were in pairs, indicating chromosome break (Figs. 6 and 13). There were some rings, minute deletions, dicentric chromosomes and chromatids.

c) Unequal length of metaphase chromosomes, showing much more variation in length than is observed in the normal karyotypes, indicating deletions, duplications and translocations.

d) Micronuclei in the interphases. These were of two different types; one group was normally circular and densely stained dots and the other type was roundish, faintly stained and having more or less a loose structural organization as of the interphase nuclei beside it (Figs. 7 and 17). The former type has been termed here as the "condensed" and the latter as the "non-condensed" micronuclei. There was increasing evidence indicating that some of these micronuclei divide (see Figs. 8, 9 and 16), and go to the daughter nuclei.

e) Degenerated cells with very little chromatin material and less stainability, sometimes obviously shrunken.

f) Giant cells (in *V. ervilia* only): These cells were about double the size of the ordinary root meristematic cells of *V. ervilia*.

Discussion

The fact that there are so many chromosomal aberrations in the irradiated samples is itself evidence that quite a large number of breaks, whether chromosome or chromatid, have occurred. As to the reunions, only the unequal exchanges under

Figs. 1-9. All figures are from root-tip cells of *Vicia ervilia*; normal chromosomes and chromosomal aberrations following gamma irradiation of seeds. 1, a somatic metaphase stage showing 14 chromosomes in an untreated control plant. 2, metaphase with fragments (50 Krads). 3, anaphase with criss-cross bridges (10 Krads). 4, anaphase with possibly interlocked bridges, fragments and laggards (30 Krads). 5, anaphase with multiple bridges, fragments and laggards (25 Krads). 6, anaphase with a pair of laggards (30 Krads). 7, interphase with "condensed" and "non-condensed" micronuclei (50 Krads). 8, micronuclei indicating possible entry into a very early prophase stage along with the main nucleus (15 Krads). 9, one of the micronuclei has possibly entered the prophase stage along with the main nucleus (20 Krads). $\times 1875$.

Figs. 10-17. All figures are from root-tip cells of *Lathyrus sativus*; normal chromosomes and chromosomal aberrations following gamma irradiation of seeds. 10, a somatic metaphase stage showing 14 chromosomes in an untreated control plant. 11, chromosome fragments left out of the equatorial plate. 12, anaphase with criss-cross bridges, fragments and micronuclei. 13, anaphase with bridges, a ring, fragments and laggards. 14, anaphase with paired bridges and laggards. 15, "U-shaped" fragment has been incorporated into the interphase cell without being straightened out. 16, the micronucleus has also possibly entered the prophase stage along with the main nucleus. 17, interphase with both "condensed" and "non-condensed" micronuclei. $\times 1875$.

new orders of arrangement can be identified due to the presence of short or long arms etc., but reunions in the original order are difficult to detect. Carlson (1941) working with X-irradiated grasshopper chromosomes suggested that restitutions might be detected in the metaphase chromosomes by the presence of constrictions in them. But he stated that the constrictions might also have been a result of partial breaks or incomplete fusions, and as certain chromosomes normally have secondary constrictions it is very difficult to differentiate between the groups for statistical analysis.

The different types of mitotic bridges detected in the present study occurred in different ways. The single bridges arise from breaks when both chromatids of a chromosome are broken at the same locus, lateral fusion occurs (Sax 1940) and dicentric and "U-shaped" acentric fragments are formed. The dicentric fragment is pulled equally to both side at anaphase and a bridge is formed. According to Sax (1940), the "U-shaped" fragment tends to straighten out but in one case in the present work it retained its "U-shape" and was incorporated into the interphase cell as such (Fig. 15).

The formation of paired bridges in the mitotic anaphases were described by Sax (1940) and Caldecott and Smith (1952) to be a result of fusion between broken chromosomes rather than broken chromatids. They concluded that the X-ray induced bridges were a result of union between two centric fragments. Prasad (1965) and Sinha (1967) also found paired bridges in the shoot and root-tip cells of irradiated samples of *Phalaris* and *Lens*, respectively.

Triple and mutiple bridges are formed depending upon the number of chromosomes involved in the breakage and followed by exchanges. Sax (1940) has suggested that when fusion occurs between the broken ends of terminal deletions, a fragment consisting of parts of two chromosomes is the result. The relational coiling between the chromatids of the dicentric chromosome persists to metaphase and during the separation at anaphase the dicentric chromatids might be separated easily, or form an interlock situation or form an X-shaped (criss-cross) bridge.

The centric fragments may unite as has been mentioned above. The centric fragments obtained in *V. ervilia* and *L. sativus* were mostly paired and showed little tendency to poleward movement. Carlson (1938) stated that acentric fragments resulting from breakage of chromosomes due to radiation tended to be lost from the daughter nuclei (due to the lack of centromere) because they move more slowly toward the poles than the normal chromosomes do. Sax (1941) from his root-tip studies with X-irradiated dormant seeds and bulbs of *Allium* concluded that acentric fragments do not become a part of the daughter nuclei.

The pairing of fragments observed in the two species of this experiment points to the fact that they have originated from chromosome rather than chromatid breaks (Caldecott and Smith 1952), whereas the multiple and the non-paired fragments observed in them suggest chromtid breaks.

Acentric fragments, dicentrics, minute deletions and rings were also found by Rana and Swaminathan (1967) in wheat and Avanzi *et al.* (1966) found chromatid type aberrations in their seed irradiation studies in *Triticum durum*.

The unequal length of metaphase chromosomes observed in this study is in conformity with the findings of Sinha (1967) in *Lens* who has suggested the possibility of asymmetrical exchange as an explanation. Sax (1940) suggested that a reciprocal interchange between the arms of different chromosomes is the reason for inequality.

Presence of micronuclei in the interphase cells is to be expected as there were many acentric fragments in the metaphases and laggards in the anaphases. Micronuclei have been reported by Clowes (1964) to be formed as a result of exclusion of the acentric fragments of chromosomes out of the nuclear membrane during the completion of mitosis. The terms "condensed" and "non-condensed" have been denoted for the first time to micronuclei by the present authors due to the obvious differences between the two groups of micronuclei in structure, thickness of chromatin material and stainability. The "non-condensed" micronuclei are formed from several chromosomes or fragments. But the increasing evidences, suggesting their ability to divide with the nucleus, are furnished by their entering a prophase condition at the same time as the main nucleus (Figs. 9 and 16). It may be possible that they become "condensed" after one division and some of the "condensed" micronuclei that are observed have already passed such a division.

The degenerated cells may probably be the cells which had too much chromosomal breakages and loss of chromatin material rendering it unable to live and function normally.

The giant cells were obtained in *V. ervilia* only. Extra large cells were also obtained by Gray and Scholes (1951) in irradiated *Vicia faba* roots. They suggested the reason of these cells being abnormally large to be their deficiency in nuclear material and consequent inability to divide and form two normal daughter cells. Tolmach and Marcus (1960) also suggested that the giant cells may result from the ultimate failure of the cell division process. The mechanism of giant cell formation, however, has not yet been explained thoroughly (Ishihara and Kumatori 1966).

Summary

The present study deals with the mitotic consequences of gamma radiation induced chromosome breaks in *L. sativus* and *V. ervilia* following seed irradiation. Various types of mitotic anomalies were encountered. These included:

1. Single, double, triple, multiple, X-shaped and interlocked bridges. The double bridges lasted very long persisting upto the late telophase and often left pear-shaped projections in the daughter nuclei marking their positions.
2. Centric and acentric fragments in the metaphase and laggards in the anaphase were present. The fragments were of different lengths and the majority of them were in pairs. There were also some rings, minute deletions, dicentric chromosomes and chromatids present.
3. Unequal length of metaphase chromosomes showing much more variation in length than is observed in the normal karyotypes.

4. "Condensed" and "non-condensed" micronuclei in the interphase cells were observed and there was increasing evidence indicating that some of these micronuclei divide and go to the daughter nuclei.

5. Degenerated cells having little chromatin material and showing less stainability were present.

6. Giant cells often having about double the size of the ordinary root meristematic cells were present only in the species *V. ervilia*.

Probable causes of the production of mitotic abnormalities following seed irradiation treatment were discussed in the light of the findings of other workers.

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