Radiation Studies in *Lens culinaris*: A Comparative Study of Meiotic Aberrations Induced by Acute and Chronic Gamma Radiations¹

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The meiotic abnormalities induced by acute ionizing radiations have been reported in earlier investigations (Mather 1934). Sinha and Godward (1972) and Acharia (1972) studied the different types of chromosomal aberrations exhibited during meiosis of *Lens culinaris* raised from the irradiated seeds. The effect of chronic radiation on *Lens culinaris* is being reported for the first time. These aberrations are compared with those caused by acute radiation.

The cells undergoing meiotic division show bipartite nature of their chromosomes from early zygotene to the end of second metaphase. The chromatid aberrations and physiological effects (surface stickiness of the chromosomes) are two abnormalities exhibited by the chromosomes when the cells are irradiated during the process of their division (Marquardt 1938, Lea 1955, Swanson 1965). Whereas, the cells which enter division after they are exposed to acute radiations, are capable of exhibiting only structural changes induced in their chromosomes (Lea 1955).

Experimental Work

Dry, dormant and pureline seeds of *Lens culinaris* belonging to both small seeded and large seeded varieties were taken for investigation. Some seeds were exposed to acute gamma radiation of 3, 6, 9, 12, and 15 KR. The irradiated seeds were then transferred to different petridishes lined with moist filter papers.

A light source of 40W Phillips Incandescent Lamp and temperature of $25 \pm 2^\circ\text{C}$ were maintained in order to get the seeds properly germinated. After germination, the seedlings were transferred to different earthen pots filled with soil and manure. All the pots were kept in the natural conditions inside a wire house.

For chronic treatment, the remaining untreated seeds were germinated in the laboratory conditions as above and were transferred to earthen pots filled with soil and manure. When the seedlings became one week old, the pots were kept in the "Gamma garden" erected at the Fertiliser Corporation of India, Sindri, Bihar, having a radioactive source of Co^{60} .

The pots were kept for 75 days in the gamma garden and placed in three different concentric arcs at the distance of 3.05, 4.57, and 6.10 meters from the radiation source which received the dose at the rate of 273.34, 166.67 and 106.67 rads per day respectively. The total radiation dose received by the nearest, middle and farthest

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arcs came upto 20.5, 12.5 and 8.0 KR, respectively.

For meiotic study, healthy flower buds were picked up and fixed in aceto alcohol (1:3) supplemented with a little ferric chloride, which acted as mordant. Anthers were squashed in 2% aceto carmine preparation and the chromosomal aberrations were recorded from 200–300 well-spread meiotic plates.

The pollen sterility was studied by staining the grains with 0.2% acetocarmine preparation. The deeply stained pollen grains were regarded as fertile, whereas, the feebly stained or unstained were treated as sterile ones. The above studies were made for different treatments separately.

Observations and results

The regular formation of seven bivalents was recorded in the normal plants of both small seeded and large seeded varieties. However, they differed from each other as regards the number of rings and rods. The number of ring bivalents ranged from 3 to 6 with the corresponding number of rod bivalents i.e. 4 to 1.

Figs. 1–3. All figures at $\times 1600$. 1, metaphase I showing fourteen univalents (acute radiation). 2, metaphase I, showing heterogeneous staining and desynapsis due to chronic radiation (arrow). 3, metaphase I, showing clumped configurations of fourteen chromosomes due to chronic treatment.

The nature of chromosomal aberrations in acute and chronic radiations were different from the very beginning as corroborated by Figs. 1–3. It was interesting to note that the chromosomes, in the case of acute treatment, were stained homogeneously throughout their surface with acetocarmine. Whereas, the chromosomes treated with chronic radiations exhibited some regions which were very feebly stained in comparison to the other deeply stained areas. It was even more interesting to note that the magnitude of injury of different aberrations were dissimilar in both the cases at different doses.

The percentage of cells with univalents at metaphase-I, were found to be less at lower dose in the case of acute irradiation. Such cells recorded an increase with the increase of doses and a maximum frequency was recorded at 9 KR dose in both the small seeded and large seeded varieties. The univalents decreased at the highest dose (15 KR) as compared to 9 and 12 KR. But, the values were higher than those at 3 and 6 KR. Whereas, in the case of chronic treatment, the cells with

univalents appeared in largest number in the plants belonging to the outermost arc (8.0 KR). The plants of the middle (12.5 KR) and innermost (20.5 KR) arcs recorded lesser number of such cells (Fig. 7).

The univalents, in the case of acute treatment, were the outcome of the failure of homologous pairing of chromosomes (Fig. 1). But, the univalents, which were recorded in the case of chronic exposure, were laying beside their respective homo-

Figs. 4-8. (In both small and large seeded varieties) 4, percentage of PMC with trivalents and tetravalents at metaphase I, in different doses of acute radiation. 5, percentage of PMC with bridges, laggards and bridges associated with laggards at anaphase/telophase I, in different doses of acute radiations. 6, percentage of PMC with trivalent, tetravalent and multivalents at metaphase I, in different doses of chronic radiation. 7, percentage of PMC with univalents and sticky chromosomes at metaphase I, in different doses of chronic radiation. 8, percentage of PMC with bridges, laggards, and bridges associated with laggards at anaphase/telophase I, in different doses of chronic radiation.

logous partner. There was no chiasma formation between them (Fig. 2).

The percentage of cells containing trivalents was directly proportional to the total acute dose received, whereas, the trend was reverse in the case of chronic irradiation (Figs. 4 and 6). As regards the number of cells possessing tetravalents, multivalents and sticky chromosomes showing clumped configurations, the order was on the increase with increasing dose. These aberrations achieved their maximum values at the highest dose received in both types of treatments. The tetravalents and multivalents were both ring and chain types with zigzag or open configurations.

At anaphase/telophase I single or multiple bridges were observed with or without fragments. In both types of treatments, the single bridges outnumbered the multiple ones at lower dose level, but at comparatively higher doses it was found to be vice versa. The percentage of cells showing bridges associated with fragments went on increasing with the increase of dose in acute radiation, whereas such cells were inversely proportional to the total chronic dose received. With the increase in total acute dose, the number of laggards increased. But, in the case of chronic irradiation, the number of laggards became lesser (Figs. 5 and 8).

At anaphase/telophase II stage, the bridges alone or accompanied with fragments showed the same trend as their respective anaphase/telophase I in two different types of treatment. The monads, diads and triads at tetrad stage were recorded to go on increasing with corresponding higher doses in both acute and chronic irradiations. As regards the percentage of cells with micronuclei, the unequal pollen grains and the percentage of sterile pollen grains, it varied from dose to dose. These abnormalities appeared to have followed a linear scale with the corresponding dose of acute and chronic irradiations.

Discussion

In earlier investigations (e.g. Alberts and Politzer 1923) it has been shown that all types of chromosomal aberrations produced in the cells which are already in the process of their division at the time of irradiation, are due to the physiological effects in contrast to the structural changes i.e. breakage and reunion of the broken parts produced by the acute exposure of the cells at their resting stage. The physiological effects are exhibited by surface stickiness of chromosomes, clumped metaphase and bridges at anaphase.

According to Darlington (1942), the stickiness in the chromosome surface is due to the alteration in the surface properties of chromosomes covering its whole surface, and the cause behind the alteration in this property is the depolymerization of the matrix of nucleic acid deposited over the chromosome surface. From the present experiment, (Figs. 2 and 3), it was clear that the chronic irradiation has certainly altered the surface properties of chromosomes which led to form the clumped configuration. But, from the heterogeneous staining of the chromosome surface, which was a remarkable abnormality here, it appeared that the depolymerization of nucleic acid was accompanied with some major injuries inflicted upon them, which might have impaired their efficiency.

The difference in the nature of the types of chromosomal aberrations caused by acute and chronic radiations underlined the fact that acute radiation was given to the cells when almost all of them possessed the chromosomes in monopartite i.e.—unsplit, condition. Whereas, the chronic radiation was applied to the cells during the process of their growth and proliferation. Under such conditions, the chromosomes were likely to undergo various types of changes as regards their morphology and function. Here, they did not behave like a sitting duck to the gamma rays, as they did in the case of acute treatment. The damages caused to the chromosomes were of a continuous nature and thereby giving rise to aberrations basically different from those recorded in acute treatment. The different biological effect of acute and chronic gamma radiations could also be attributed to the fact that in the case of acute treatment, the cells under investigation reached that stage after thousands of proliferations and accordingly, rigorous selection mechanism had fully operated. Due to this, the cells with drastic changes and irreparable damages got eliminated and only tolerable structural changes were observed in majority of the cells. Hence, comparatively stable conditions were expected. Whereas, in chronic radiation the cells were being directly hit and the damaged cells with drastic changes were observed.

In both types of treatments, the univalents were recorded. But, in the case of acute irradiation, the univalents were not found to form an apparent bivalent with its homologous chromosomes (Fig. 1). Hence, the univalents in this case were the outcome of asynapsis. Whereas, in chronic treatment, the univalents were laying beside their respective homologous partner, but there was no chiasma formation between them (Fig. 2). This sharply underlined their desynaptic nature. The initial increase in the number of the cells containing univalents with increasing acute dose, suggested that there had been a mutation in the gene controlling the pairing and subsequent formation of chiasmata between homologous chromosomes (Venketaswaralu and Rao 1968, Sinha and Godward 1972). Such abnormal behaviour might also be the outcome of the tolerable changes induced by acute radiation at intrachromosomal level in the embryonic stage. But their decreasing tendency towards the still higher doses was possibly due to the increasing translocations between the non-homologous chromosomal segments leading to the formation of trivalent, tetravalents and multivalents in ascending order.

The mutation of the gene controlling the chiasma formation after the apparent pairing of homologous chromosomes might have been the possible factor behind the univalent formation in the case of chronic treatment. Here, the decreasing tendency in the percentage of pollen mother cells possessing the univalents with the corresponding increase in the total dose received, seemed to indicate that at lower doses, the lesser degree of surface stickiness was produced giving rise to their independent status. As the dose increased, the sticky nature enveloped the entire surface of the chromosome to get attached with any other chromosomes forming multivalents and thereby decreasing the number of univalents.

The alteration in the surface properties and reciprocal shifting of chromosomal segments went on increasing with the increase of dose and resulted in the formation of greater number of bridges at anaphase-I in both types of treatments. However,

the decreasing tendency of the number of fragments associated with such bridges in the chronic treatment could be due to the ascending degree of surface stickiness and their subsequent association with other clumped chromosomes. The disturbances caused to the normal spindle formation seemed to be the possible cause behind the laggard formation (Sinha and Godward 1972). Their increasing or decreasing numbers with the corresponding increase in the acute and chronic doses respectively deserved the same explanation as the fragments explained above.

The linearity of the percentage of monads, diads and triads with the increasing dose in both acute and chronic treatments suggested unequal distribution of the chromosomes to daughter nuclei and subsequent failure of wall formation at certain stages. The same might have been the possible cause of the formation of unequal pollen grains. The occurrence of pollen sterility and their increasing order with the increasing dose in both treatments might have been due to the cumulative effects of the different types of aberrations noted in two different cases.

In the present investigation, it was not possible to compare the nature and trends of different types of aberrations from a common dose level of the two types of treatments. But seeing the overall tendency of different types of aberrations, it was clear that the chronic radiation caused more drastic changes as compared to that in the acute treatment. However, it needs further investigation.

Summary

The large seeded and small seeded varieties of *Lens culinaris* were given acute and chronic exposure of gamma radiations. Their chromosomal aberrations during microsporogenesis of M_1 generation were compared.

In the chronic treatment, the univalents, trivalents, tetravalents, laggards and fragments registered a decreasing trend with the increasing radiation dose. Whereas, all the aberrations except univalents were found to go on increasing with the total acute dose received. The univalents here showed a depression at the highest dose.

The other aberrations viz. tetravalents, multivalents, cells with clumped configurations, bridges at the anaphase/telophase, unequal pollen grains, and pollen sterility were on the increase at the corresponding higher doses. This was common in both types of treatments. The physiological effects in the case of chronic treatments was mainly attributed to the existing differences from the plants treated with the acute radiations.

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