The Irradiation Sensitivity of Barley Seeds Low in Calcium[1]

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CONSIDERABLE EVIDENCE indicating a direct connection between calcium and magnesium metabolism and chromosome structure has now accumulated. Biochemical studies by Bernstein and Mazia (1953) and Mazia (1954) showed that chromosomes could be dispersed into Fuelgen sensitive particles by treatment with chelating agents in a suitable ionic environment. On this evidence Mazia (1954) concluded that the chromosome is a particulate structure composed of macromolecular complexes of nucleic acid and proteins linked by bonds of divalent ions.

Subsequent research has further indicated that calcium and magnesium are integral constituents of the chromosome. Steffensen (1953, 1955) found evidence of chromosome breakage in *Tradescantia* plants grown on nutrient media deficient in calcium and magnesium. More recently and after the present study was completed Steffensen (1956) reported that suboptimal calcium increased the sensitivity to X-rays of the chromosomes in *Tradescantia* microspores. Levine (1955) reported a decrease in crossing over in *Drosophila* resulting from excess calcium in the diet of adult females. He noted increased genetic recombination if the chelating agent ethylenediamine tetra-acetic acid (versene) was added to the culture medium. Eversole and Tatum (1956) found similar increase in crossing-over after applying versene or $MnCl_2$ to *Chlamydomonas.* Hyde (1956) reported that *Vicia faba* chromosomes treated with versene swelled and tended to lose definition.

The present study was designed to determine the level of spontaneous chromosome aberrations and to assess the sensitivity of chromosomes to X-rays and thermal neutrons in seeds derived from plants grown on a substrate deficient in calcium.

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Materials and Methods

Diploid barley, *Hordeum vulgare* variety Himalaya, was grown in quartz sand and subirrigated twice a day with modified Hoagland's solution. The Hoagland's solution was standard except that the calcium concentration was 0.1 of standard. To insure an optimal supply of elements, other than calcium, the solution was changed every three weeks from planting time until the flag leaf appeared in a majority of the plants. The seeds were harvested at maturity.

A spectrophotometric analysis by the Industrial Research Division of the State College of Washington showed that the seeds from plants cultured on the suboptimal calcium solution contained 2,500 ppm. of calcium. Seeds grown under standard conditions contained 10,000 ppm.

Samples of the calcium-deficient and control seeds were X-irradiated at Pullman, and X-irradated and exposed to a thermal neutron flux at Brookhaven National Laboratory.

The seeds treated at Pullman were stored in a desiccator over $CaCl_2$ for six weeks so they contained 8 per cent moisture at the time of irradiation. They received 15,000 *r* of unfiltered X-irradiation from a beryllium window A.E.G. Machlett X-ray tube operated at 34 KV and 25 ma. Additional details of irradiation treatments of resting seeds and of the X-ray apparatus have been published (Adams and Nilan, 1957).

At Brookhaven, separate samples of the calcium-deficient and control seeds received an X-ray dose of 7,500 *r* and a total neutron dose of 4.22×10^{12} $cm^2 \times sec^{-1}$. Additional details of the irradiation treatments at Brookhaven have been described earlier (Caldecott, 1955).

Each irradiation treatment was administered in duplicate.

Both X-ray and neutron-treated seeds were germinated on blotting paper. After fixation in Carnoy III (3 parts ethanol, 4 parts chloroform, and 1 part acetic acid), acetocarmine smears of shoot tips were examined cytologically for dicentric chromosome bridges and acentric rod and isodiametric fragments at late anaphase of the first cycle of mitosis. Photomicrographs of such aberrations may be seen in Caldecott and Smith (1952).

Experimental Results

The frequencies of dicentric chromosome bridges and acentric rod and isodiametric fragments of the various treatments are recorded in Table 1.

The data reveal that an appreciable frequency of chromosome aberrations can be attributed to calcium deficiency alone. This increase was observed for both the dicentric bridges and the two types of fragments.

TABLE 1. FREQUENCIES OF CHROMOSOME ABERRATIONS IN CALCIUM-DEFICIENT BARLEY SEEDS NONIRRADIATED, X-IRRADIATED, AND THERMAL NEUTRON TREATED

(*Summary of two replications*)

Calcium Level	Irradiation	Cells	Dicentric Bridges/ Cell	Rod Fragments/ Cell	Isodiametric Fragments/ Cell
Normal	O *r*	400	.012	.01	.002
Deficient	O *r*	400	.06	.052	.117
	X-ray				
Normal	7,500 *r*	400	.23	.43	.73
Deficient	7,500 *r*	400	.38	.63	1.36
Normal	15,000 *r*	400	.32	1.13	2.23
Deficient	15,000 *r*	400	.64	1.60	3.19
	Thermal neutron				
Normal	$4.22 \times 10^{12}/cm^2 \times sec^{-1}$	396	.46	.76	1.57
Deficient	$4.22 \times 10^{12}/cm^2 \times sec^{-1}$	400	.41	.73	1.70

It is also apparent that the calcium-deficient seeds were more sensitive to X-rays than the control seeds. The increase in chromosome aberrations found in the low-calcium seeds was much greater than could be explained by spontaneous aberrations owing to calcium deficiency *per se.*

Low-calcium level, however, had no effect on the chromosome-aberration frequencies induced by thermal neutrons. Neither bridge nor fragment frequencies increased appreciably in the seeds deficient in calcium. Slight differences between frequencies in favor of the calcium-deficient seeds could be attributed to the spontaneous aberrations already present in the calcium-deficient seeds.

Discussion

This study has shown that seeds deficient in calcium exhibit an appreciable frequency of chromosome aberrations. Furthermore, the chromosomes in these seeds are more sensitive to breakage by X-irradiation than those in normal calcium seeds. Nevertheless, they exhibit no change in sensitivity to breakage by thermal neutrons.

Parts of these findings are similar to those reported by Steffensen (1953, 1955, 1956) in *Tradescantia* plants raised on suboptimal calcium and magnesium culture media. He concluded that calcium and magnesium are involved with complexing and binding of chromosomal nucleoprotein and, thereby, are a part of the structural constitution of the chromosome. Furthermore, he maintained that calcium and magnesium form bonds between DNA molecules at key sites along the chromosome. It follows that calcium deficiency would weaken or disrupt these sites. The spontaneous aberrations would arise from complete disruption of these sites.

Based on this structural hypothesis it is possible to explain the difference in sensitivity of the chromosomes in the calcium-deficient seeds to X-rays and thermal neutrons. The sparsely ionizing X-rays would be much more effective in breaking a weakened site than an unaltered portion of the chromosome. The densely ionizing and more efficient neutrons, on the other hand, would cause chromosome breaks with equal frequency at a weakened site or at an unaltered portion of the chromosome. Steffensen (1956) used a somewhat similar hypothesis to explain the X-ray sensitivity of chromosomes in calcium-deficient *Tradescantia* inflorescences.

The results of this study add to the growing body of evidence (Giles, Beatty, and Riley, 1952; Nybom, Gustafsson, and Ehrenberg, 1952; Ehrenberg, Gustafsson, Lundqvist, and Nybom, 1953; Caldecott, 1956) indicating that the biological effects of neutrons and X-rays cannot be modified or altered to the same degree.

The results also indicate how spontaneous chromosome aberrations and, presumably, mutations might arise in nature. Many soils are low in calcium. Plants growing on these soils would produce seeds reduced in calcium. These seeds, in turn, would contain spontaneous cytogenetic changes. Such changes would be then passed on through the plant population and would provide genetic variability.

This study also suggests an explanation for the cytogenetic changes that occur in aged seeds (Gunthardt *et al.,* 1953; D' Amato and Hoffmann-Ostenhof, 1956). It has been shown that metabolic waste products such as putrescine (tetramethylenediamine) and cadaverine (pentamethylenediamine) are both mutachromosomic and mutagenic (D'Amato, 1952; D'Amato and Hoffmann-Ostenhof, 1956). Water extracts of such decomposition products have induced chromosome aberrations (Mota, 1952). Since many of these metabolic wastes are strong chelating compounds, it is not unreasonable to suspect they would complex Ca^{++} ions in the aged seeds, thereby causing chromosome aberrations such as those described above. This hypothesis has been used earlier to account for the increased sensitivity of chromosomes to X-rays in aged seeds (Nilan and Gunthardt, 1956).

Summary

Frequencies of chromosome aberrations were determined for un-irradiated, X-rayed, and thermal neutron-treated normal and calcium-deficient barley seeds. Calcium-deficient seeds exhibited appreciable frequencies of chromosome aberrations and the chromosomes were more sensitive to X-radiation. However, the chromosomes did not respond any differently from those in the normal calcium seeds to thermal neutron treatment. The spontaneous aberration data support the hypothesis of Steffensen that calcium is an important structural constituent of the chromosome. The differences in the action of X-rays and thermal neutrons are explained on the basis of ion density and efficiency of each type of radiation.

The results of this study are discussed in relation to the cytogenetic changes that occur spontaneously in plants and arise in aged seeds.

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