MODIFICATION OF LEAF STRUCTURE BY X-RAYS

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(WITH SIX FIGURES)

Introduction

The effect of x-rays upon seeds and seedlings very often causes abnormalities of form and changes in the internal structure of the plant. The cells of a treated radicle show elongation, great vacuolation or entire lack of protoplasm, and absence of nuclei from many cells (**1, 6**). Strong irradiation sometimes causes tumor-like tissues at the root tip, which give a bulbous and swollen appearance and which contain multinucleated giant cells (**4, 7**). Fasciations of stems, leaves, and flowers in the sunflower and bushy forms of young tomato plants occur after treatment (**1, 3**). Abnormality of leaves is a very common injury by x-rays: the leaves become asymmetric, distorted, pocked; light green areas intermingle with ordinary green as if the leaves were suffering from a mosaic disease (**1, 3, 10**).

Very few studies have been made on the structure of these abnormal leaves. The writer found that one investigator has reported the interference with the normal development of palisade cells and spongy parenchyma which takes place in such leaves (**3**).

Material and methods

Since Russian sunflower, *Helianthus annuus,* is very sensitive to x-rays and abnormality of vegetative parts can easily be produced by irradiation, this plant was selected for these experiments. Seeds were placed for about 24 hours in a moist chamber on a layer of cellucotton saturated with distilled water, and kept at a temperature of 25° C. Some investigators (**2, 5, 8, 9**) found that the sensitivity of seeds to x-rays is influenced by the amount of water they contain at the time of treatment; therefore it is necessary to measure the water content of the material. The average water content of air-dried seeds of *Helianthus* is 3.75 per cent. After keeping them in moisture for 24 hours this increases to 38.83 per cent. and the seeds show incipient germination. At this stage, after removing the pericarps, as uniform seeds as possible were selected for treatment and controls. The irradiation used in these experiments was made with an x-ray machine in the following set-up: 100 K.V. 5 ma. current, no screen, 30 cm. focal distance. Immediately after treatment both irradiated seeds and controls were planted in pots and kept under environmental conditions as much alike as possible, in the greenhouses of the University of Chicago.

Results

General aspects of leaf abnormality

The most common deformities presented by leaves after treatment are asymmetry of blade and distortion caused by small pockets. Light or dark green (occasionally both) areas intermingle with ordinary green, and, very rarely, even colorless portions appear along the margin of the leaf (fig. 1, *a–c*). With strong irradiations the first two or three leaves show a peculiar aspect, as if they were suffering from a mosaic disease (fig. 1, *d*).

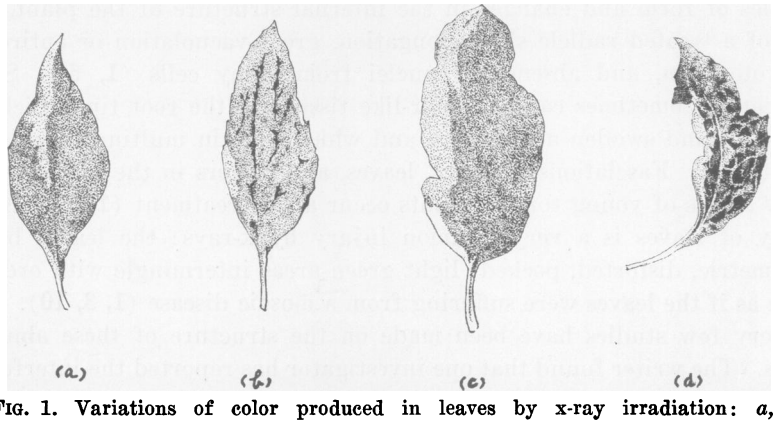

FIG. 1. Variations of color produced in leaves by x-ray irradiation: *a*, light green areas in ordinary green leaf; *b*, dark green scattered streaks; *c*, colorless area along the margin; *d*, mosaic diseased appearance.

An experiment has been performed with the time element as the only variant in the production of abnormalities in leaves. The seeds were divided into five series of ten seeds each, and irradiated for 5, 10, 15, and 20 minutes respectively, the remaining lot being unradiated, as control. Observations were made after ten weeks, when the plants were nearly mature. Table I records the abnormalities observed.

Histology of normal leaf

Cross sections of young leaves about 1.5 cm. long, from normal plants which were used as controls, were examined to determine the normal development of the tissues in the early stages. One or two layers of long but uniform palisade cells lie under the epidermis, and the space between the palisade tissue and the epidermis of the under surface of the leaf is filled with small cubic spongy parenchyma. All the cells in young tissue appear light green with compactly arranged chloroplasts. As the leaves grow the palisade cells become longer and the spongy parenchyma cells become angular in shape. The green elipsoidal chloroplasts are found

TABLE I

LEAF ABNORMALITIES APPEARING AFTER IRRADIATION OF X-RAYS: M, HEAVY MOSAIC DISEASED APPEARANCE; m, LIGHT MOSAIC DISEASED APPEARANCE; L, LIGHT GREEN AREAS INTERMINGLED WITH NORMAL GREEN; l, SMALL LIGHT GREEN AREAS INTERMINGLED; D, DARK GREEN AREAS INTERSPERSED WITH NORMAL GREEN; w, COLORLESS AREAS APPARENT; N, NORMAL

No. LEAVES	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
No. PLANTS	Series I (20 minutes' treatment)																	
1	M	M	the bud arrests growth															
2	M	M	Lw	L	l	l	ml	Ml	l									
3	M	M	L	D	l	l												
4	m	m	L	LD	l	lw	lw											
5	M	M	L	L	L	L	l	l										
6	M	M	L	L	L	L	L	L										
	All leaves asymmetric and distorted; four plants died from heavy injuries before the emergence of leaves																	
	Series II (15 minutes' treatment)																	
1	M	M	m	l	l	a side bud appears												
2	M	M	L	l	l	N	N	l	N									
3	m	m	L	L	the bud arrests growth													
4	M	L	L / N	L / N	L / ...	two buds develop												
5	m	m	L	L	l	N	l	l	l									
6	M	M	m	m	L	L	l	l	l									
7	m	L	L	N	N	N	N	N	N	N	N	N	N	N				
	All leaves asymmetric; three plants died in seedling stage																	
	Series III (10 minutes' treatment)																	
1	L	L	L	l	l	l	N	N	N	N								
2	m	m	N	N	N	N	N	l	N									
3	L	L	l	l	N	N	N	N	N	N	N	N	N					
4	L	L	l	l	l	N	N	N	N	N	N	N	N					
5	m	m	L	l	l	N	N	N	N	N	N	N						
6	M	M	m	m	L	L	l	l	l	N	N	N	N	N				
7	m	m	D	d	N	N	N	N	N	N	N	N	N					
8	m	m	m	L	L	l	l	N	N	N								
9	M	M	L	L	l	l	l	N	N									
	All leaves slightly asymmetric and pocked; one plant injured by insects is excluded from the table																	
	Series IV (5 minutes' treatment)																	
1	m	m	l	l	N	N	N	N	N	N	N	N	N					
2	m	m	N	N	N	N	N	N	N	N	N	N						
3	m	m	l	N	N	N	N	N	N	N	N	N	N	N	N			
4	L	L	N	N	N	N	N	N	N	N	N	N	N					
5	m	m	m	m	N	N	N	N	N	N	N							
6	m	m	N	N	N	N	N	N	N	N	N	N	N					
7	m	m	L	L	L	l	l	N	N	N	N	N	N					
8	m	m	l	l	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	All leaves symmetric; two plants injured by insects																	

gathered closely along the inside cell wall. In the mature leaf (4 cm. in length) the arrangement of cells is very regular.

Histology of abnormal leaf

Colorless part.—The appearance of colorless parts is rare; it seems to be a severe injury since it was induced only in a series of strong irradiation. The structural aspect of the leaf is just like that of an ordinary variegated leaf. There is no disturbance of tissue in the green part, although the color is somewhat lighter, probably owing to the defective chlorophyll-absorbing power of the chloroplasts. On the boundary between colorless and green tissues, in most cases the abnormal development of both palisade and spongy cells is found in the colorless area. The palisade cells are, in some instances, unusually slender, frequently curved, smaller, and more spherical. Discrepancies often occur in place of the regularity of the normal palisade arrangement. No differentiation between palisade and parenchyma occurs in the center of the colorless section when x-ray dosage is severe. A few chloroplasts without chlorophyll are found in cells of colorless tissue.

Light green part.—Two kinds of structures are found in the light green section of the leaf. Sometimes the color is caused by the small quantity of chloroplasts in the cells; in such cases no change of structure occurs. Usually, however, the development of tissues, especially palisade, is abnormal, accompanied by a lack of chloroplasts. In the normal leaf palisade cells stand side by side very compactly, but in the abnormal leaf, owing to the irregular arrangement, small or large spaces appear between palisade cells (fig. 2). When the injury is considerably severe, the entire

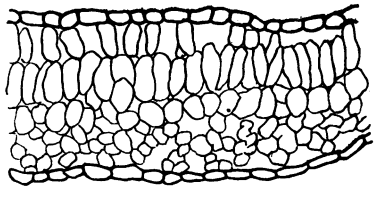

Fig. 2. Abnormal structure of light green parts. Small or large spaces appear between palisade cells owing to their irregular arrangement.

leaf appears light green with small irregular streaks of normal or dark green scattered over the surface, and the texture of the leaf becomes exceedingly coarse. Significant interference of development between palisade and spongy parenchyma is indicated in the light green areas of such leaves (fig. 3). The chloroplasts in the abnormal cells are more spherical and smaller than in the normal; they also seem to lack the normal power of absorption of chlorophyll.

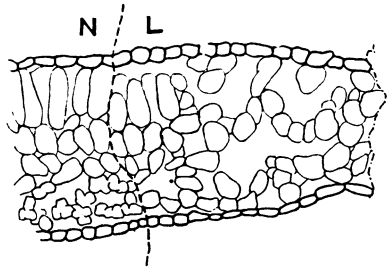

FIG. 3. Strong interference with development of palisade and parenchyma in the severely injured light green area: N, normal; L, light green parts.

DARK GREEN PART.—Dark green irregular streaks appear scattered over the leaf surface. This deep color is due to the extremely strong chlorophyll-absorptive power of the chloroplasts.

MOSAIC-DISEASED APPEARANCE.—The most interesting leaves were those showing the mosaic-diseased appearance. Distinct disturbance of development occurred in all tissues, and when the cross section of the leaf was observed under the microscope, unusual structures were frequently found. The arrangement of epidermal cells was more or less wavy or zigzag. Within a single microscopic field of vision the cells ranged from dark to normal to light green in color (fig. 4). Occasionally one or two layers

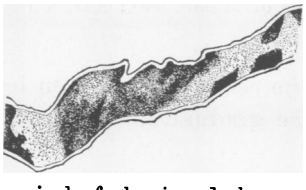

FIG. 4. Cross section of mosaic leaf showing dark, normal, and light green areas.

of colorless cells were found directly beneath the epidermis. From these observations it is readily concluded that the structure of the abnormal leaf is very complicated, and even in the least disfigured leaf several structural abnormalities will be found.

The general aspects of structure of the light and normal green areas are the same as those already mentioned (fig. 5, left, middle). The structure of the dark green areas, however, is entirely different from that of the dark green area described above.

The distinction between palisade and parenchyma tissues in most cases no longer exists. The deformed chloroplasts, clustered in groups of approximately six to eight, fill the cells and are a more bluish green. Frequently, moreover, the chlorophyll diffuses through the cell sap; thus the leaf appears even darker. The walls of the parenchyma cells are thin and less distinct. In the lower layer of the parenchyma coagulation of the

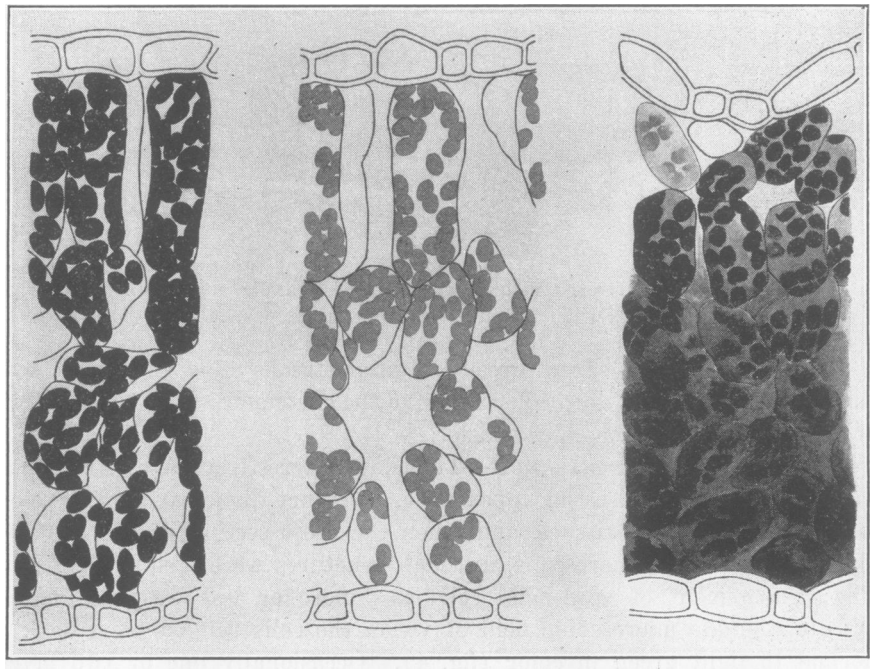

FIG. 5. Internal structures of mosaic leaf: left, normal; middle, light green; right, dark green portions.

chloroplasts occurs and the cell walls are even less distinct (fig. 5, right). Pockets are caused by the gradual or sudden interruption of a layer of palisade cells.

After the longest periods of irradiation a very peculiar structure is found in the leaf with the mosaic appearance. All of the cells become

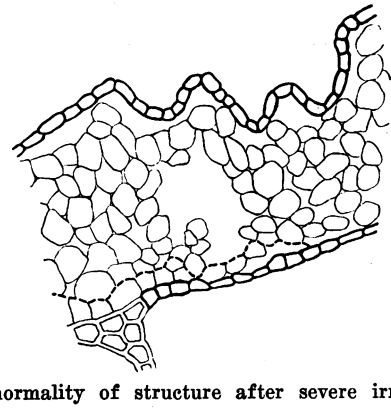

FIG. 6. Strong abnormality of structure after severe irradiation by x-rays. All the cells become globose and similar and are accompanied by one layer of colorless cells (under the broken line).

globular and similar and contain only a very small number of chloroplasts or none at all (fig. 6). The abnormality of structure can be seen even in the early stages of a treated leaf.

Effect of minimum dose of x-rays

It has been reported by several investigators that comparatively strong x-rays induce leaf abnormality, but apparently the minimum limit has not been determined. When seeds which had been imbibing water for 24 hours were treated longer than five minutes, the abnormalities appeared on the leaves. Table II summarizes the results of three experiments, including a brief description of abnormalities occurring after treatment.

TABLE II

Relation of abnormalities to length of irradiation period. Each experiment was repeated three times and twelve seeds were used in each series

No. weeks after treatment	Time of treatment			
	5 minutes	10 minutes	15 minutes	20 minutes
3	None	First two leaves show mosaic appearance	First two or three leaves like those of mosaic plants*	Severity of injury kills half the plants; abnormality severe even in early stages
4	Very slight abnormalities at tip of leaf	Abnormality comparatively severe and growth of plant irregular	Abnormalities very severe	Abnormalities very severe
5	,,	,,	,,	Growth of plant arrested by injury
6	,,	,,	,,	Very severely injured; plants die later

* One plant had leaves which showed only slight injuries.

The results given in table II show that the minimum limit is less than a 5-minute irradiation period. A further experiment was performed to determine the shortest period of treatment that would produce injury. Five series of plants which were treated 1–5 minutes showed very slight abnormalities at the tips of the leaves in the series treated for four minutes,

four weeks after treatment; the same abnormality was recognized in a series treated for three minutes five weeks after treatment.

Localization of injuries induced by x-rays

Whether the effect of x-rays upon seedlings is localized or not is one of the interesting problems connected with leaf abnormality. Three sets of experiments were performed using seeds which had been imbibing water for 1, 2, and 3 days respectively. No essential difference could be seen in treatment with seedlings of different stages, however, and only a summary of the results will be given.

In a series of experiments, germinated seeds were treated in one set with a metallic screen (3 mm. copper plate) protecting the tops and in another with the screen protecting the roots. The third series was treated without the screen. The fourth, untreated, served as a control. A remarkable difference was observed in the time taken by the seedlings to emerge from the soil. Of the seeds treated for 20 minutes, after they had been planted a week, the control showed 100 per cent. emergence, the top-screened 41 per cent., the root-screened 50 per cent., but none of the unscreened seeds emerged. Generally the root-screened seedlings emerged somewhat more rapidly than the top-screened, while the unscreened were extraordinarily retarded in emergence. Irradiation of the bud retarded growth, so that the control and top-screened seedlings showed almost the same rate of growth; but the elongation of the stem in the other two series was much retarded. The average height of the plants in the latter case is about one-half or one-third of the height of the control plants.

No abnormality appears on the leaf when the top of the plant is protected by the screen; root-screening alone has no effect in preventing appearance of top abnormality, even if the degree of injury is somewhat less than that of the unscreened seeds. The shorter the irradiation period the less the difference of leaf abnormality in the four series (control, top-screened, root-screened, and unscreened).

A complete localization of injuries by x-rays is found in the histological investigation. The incomplete development of vascular bundles in the stem, the abnormality of root cells, and the appearance of dark brown color in the hypocotyl region from the treatment by x-rays, as Johnson (1) reported, were also found and their localization observed in these experiments.

Summary

1. The seed of the sunflower is sensitive to x-rays and often shows abnormal development of vegetative parts on germination after treatment. Abnormality of the leaves is the most common symptom, however, and is constantly induced by a strong irradiation. The leaves become asym-

metrical and crumpled in appearance and develop more or less sinuous contours; they also frequently show irregular coloring of blades. Usually light, dark green, or colorless areas intermingle with ordinary green; even such a peculiar aspect as apparent symptoms of mosaic disease occurs when the injury is severe. Of course, the longer the period of irradiation of the seeds the more marked is the abnormality of the leaf.

2. The internal structure of the colorless part is like that of the ordinary variegated leaf. Cells of palisade tissue are small and slender, frequently curved, and their arrangement is disturbed by the occasional absence of cells.

3. Two kinds of structure are found in light green areas. One is normal as the light color is caused only by the small number of chloroplasts in each cell; the other shows disorder of palisade tissue with much space between cells and an accompanying lack of chloroplasts, which probably have some deficiency in their chlorophyll-absorbing power. Noticeable interference between palisade cells and spongy parenchyma occurs when the entire leaf shows light green color with small streaks of darker green.

4. In the leaves in which dark green tissue is intermingled with normal green tissue no peculiar variations can be found. The deeper color is due only to the greater accumulation of chlorophyll in the chloroplasts.

5. The structural aspect of the leaf with mosaic appearance is more complicated. The four areas, colorless, light green, dark, and normal green, intermingle throughout the leaf structure. The structure, however, is just like that of the preceding color sections except the dark green areas. Strong disturbance takes place in all tissues: the distinction between palisade and parenchyma cells disappears and the chloroplasts aggregate in groups, and sometimes coagulate, giving rise to a bluish green color. Moreover, chlorophyll diffusing out into the cell sap produces a deeper green color.

6. The minimum time limit for inducing leaf abnormality by x-rays is 3 or 4 minutes' irradiation under the following conditions of radiation: 100 peak K.V., 5 ma. current, no screen, 30 cm. focal distance.

7. X-ray injuries seem to be localized and confined to directly treated regions, and to definitely localized cellular tracts. The apparent injury and structural modifications are both localized.

These experiments were performed at the botanical laboratory of the University of Chicago. The writer expresses his thanks to Professor C. A. SHULL for his kindness in suggesting this problem and for his advice in carrying out the experiments.

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