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The Effects of Chronic and Acute Exposures of Ionizing Radiations on Kalanchoe "Brilliant Star"

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BOTANY

The Effects of Chronic and Acute Exposures of Ionizing Radiations on *Kalanchoë* "Brilliant Star"[1]

INTRODUCTION

This study was undertaken to determine and compare the effects of long term (chronic) and short term (acute) irradiation on the shoot apex of Kalanchoë. This plant was originally selected for detailed study because of its peculiar response to chronic irradiation, a study still in progress, but it was also found that its decussate phyllotaxy and almost naked shoot apex made it a convenient system for morphogenetic experimentation.

MATERIALS AND METHODS

The seeds for these experiments were obtained from the Department of Horticulture of the Michigan State University. For the chronic exposure portion of the experiment the seedlings were grown under the usual greenhouse conditions. The plants were placed in the "gamma greenhouse" around a cobalt-60 source in a circle of 80 cm radius. At that time all plants showed two true leaves; thus it can be assumed that the population was in late plastochron 2 or early plastochron 3 since at most one very young leaf pair could be hidden from the observer.

The population was sampled at weekly intervals. Five treated plants and five unirradiated controls from an adjacent greenhouse were preserved in F.A.A. (Johansen 1940), then dehydrated in a tertiary butyl alcohol series and embedded in tissuemat. The material was serially sectioned in the median plane at 10 micra and stained in safranin-fast green.

Seedlings used for the acute irradiation aspects of the experiment were placed in a controlled temperature and light chamber some

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time before treatment and removed only for the time required for the irradiation, a span of approximately 30 minutes. The temperature in the chamber was held at 75° F during the 14½ hr. day and at 65° F at night. The light source was a series of 96T8 cool light fluorescent bulbs delivering approximately 1200-1500 foot candles at one meter from the source as measured with a Weston Model 614 Photronic light meter. Two incandescent bulbs supplied the red portion of the spectrum.

The plants were measured at weekly intervals for growth in internode length and the leaf number was noted. The initial population was represented by at least ten plants for each treatment but because of disease the population decreased during the course of the experiment. However, at no time were there less than five plants per treatment.

Two types of irradiation are dealt with in this study. Both are electromagnetic and ionizing and in their action on the plant no significant difference would be expected due to any differences in their physical nature. The chronic irradiation was supplied by a cobalt-60 source of about 11 curies placed in a "gamma greenhouse". The plants here were exposed to 340 r of gamma rays per 20-hr. day, the remaining four hours being devoid of irradiation for greenhouse maintenance purposes. The acute X irradiation was applied with a G. E. Maxitron Model 250 KVP and 30 ma. with a 1 mm aluminum filter. One group of ten plants received at the apical region 500 r in 1.4 min., and another group of 20 plants 2,000 r in 5.65 min. No shielding of the rest of the plant was attempted because of the technical difficulties involved. It thus must be kept in mind that irradiation of other parts of the plant may have contributed to the effects observed. Ten plants furnished the control population. The reason for twice the number of plants in the 2,000 r group is that at this particular dose about 50% of the plants form abnormal shoot systems such as twin shoots or altered phyllotaxes and thus do not lend themselves to the type of measurement applied.

CHRONIC IRRADIATION

Observation and Discussion: Plate 1, Fig. 1, shows a sagittal section through the shoot apex of a normal plant in early plastochron 6. One leaf 5 can be seen at 90° from the young primordia of leaf pair 6. The

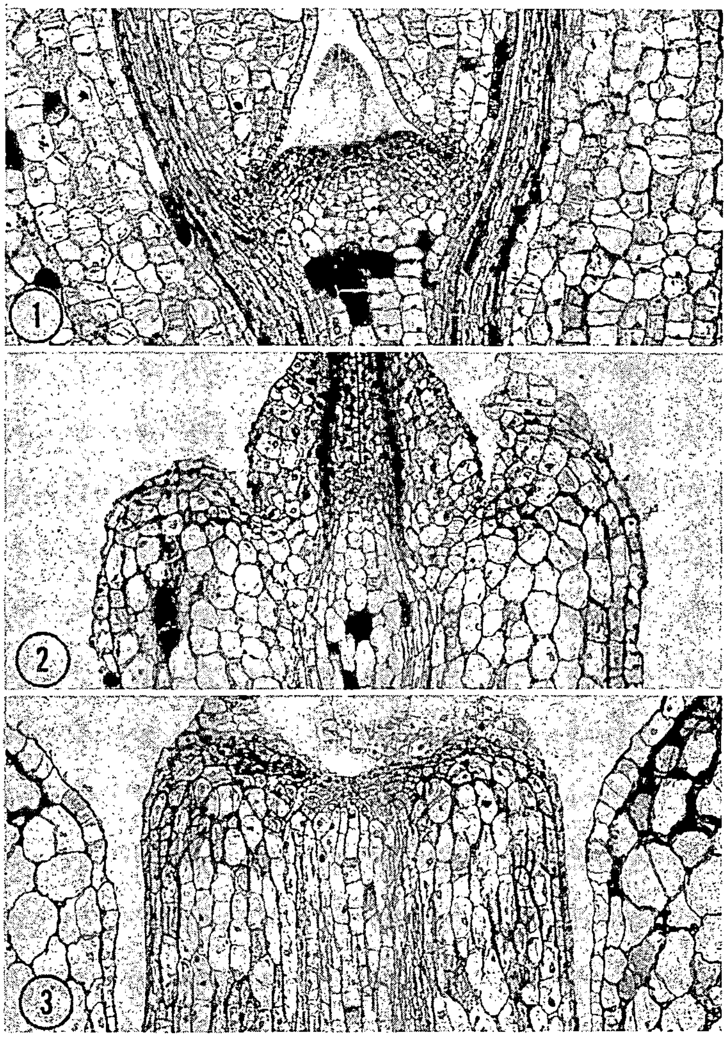

Fig. 1-3. Sagittal sections through shoot apices of **Kalanchoë** "Brilliant Star".—Fig. 1. Shoot apex of unirradiated plant in early plastochron 6. The young leaf primordia can be seen projecting beyond the outline of one of the pair of leaves 5 which are aligned at right angles to leaf pairs 6 and 4. X94.—Fig. 2. Shoot apex of plant which received 330 r/day for five days. The normal appearing leaf pair 3 is tightly appressed indicating a much restricted apical meristem. X100—Fig. 3. Shoot apex of plant which received 330 r/day for 12 days. Note complete inhibition of apical growth, though lateral growth has continued to a degree that the apical meristem proper is almost embedded in surrounding tissue. X92.

general area of the apical meristem is delimited laterally by the bulges of the most recently formed primordia, and is indicated in depth by the more densely staining cells. In median section then the meristem is some 10 cells wide and approximately four layers deep. Fig. 2 shows a median section through a plant which had been exposed to gamma irradiation for five days. Leaf pair 3 appears to be developing in a normal fashion. However, no further leaves have been produced and the meristematic region appears much restricted. This inhibition is a typical aspect of all the chronically irradiated seedlings examined so far. Of 20 plants with 12 or more days of irradiation, six showed inhibition of leaf production in leaf stage 4 and the remainder in leaf stage 3. Fifty other seedlings were examined only morphologically and these showed an average leaf stage of 3.4. Thus, probably only one and at most only two more leaves are formed after initial exposure to chronic irradiation at this dose-rate. Whether there exists a correlation between phase of plastochron at time of exposure and number of leaves produced is currently under examination. Although apical growth seems inhibited, lateral growth continues as can be seen in Fig. 3. At 12 days after initiation of irradiation the procortical region has developed beyond the height of the apical meristem which can be seen to be partially embedded by the surrounding tissue. Vascularization also has progressed well into the apical environment. Fig. 4 shows the general morphological aspect of a plant at this stage.

After approximately 30 days of exposure to irradiation, the apical meristem appears to resume its activity and produce new organs. These do not appear to arise from a shoot apex proper but rather from a general meristematic region. Many leaves may be produced in this fashion with no orderly phyllotaxy. Plate 2, Fig. 5 is a tangential section well towards the leaf attachment region as indicated by the two foliar traces at the bottom of photograph. A single leaf has been formed in this region and an adjacent meristematic region can be identified. It is also evident that vascularization has continued in not too abnormal a fashion in this case. A photograph of a section only a few 100 micra removed would still have shown some vascularization but a leaf primordium of different age and a meristematic region of different conformation. The morphology of

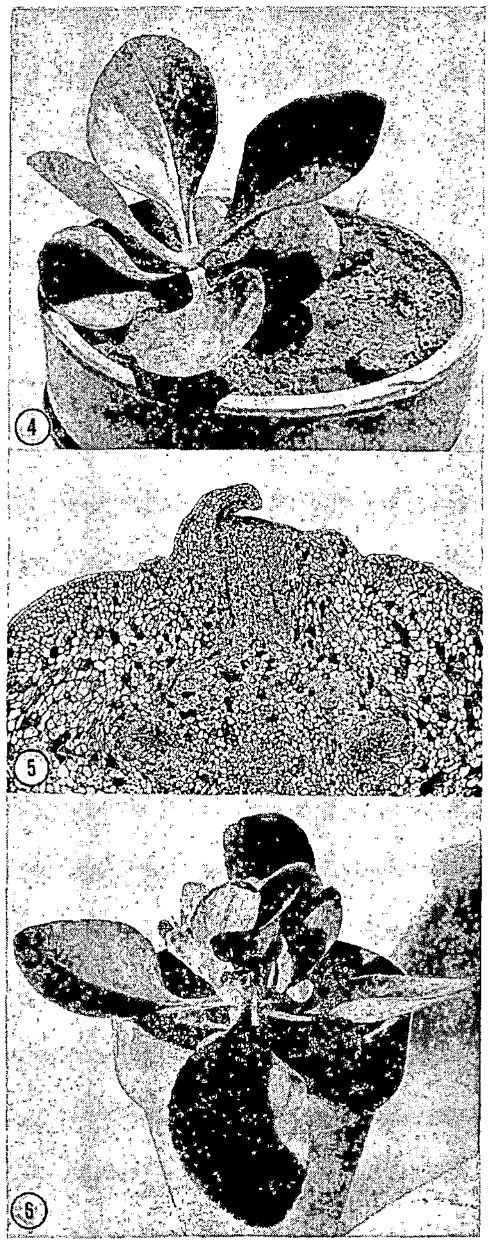

Fig. 4-6. Kalanchoë "Brilliant Star".—Fig. 4. Morphological aspect of plant similar to that in Fig. 3. Note broadened apical region and bulging leaf bases.—Fig. 5. Tangential section through apical region of plant subjected to 33 days of irradiation. Note somewhat abnormal appearing single leaf and adjacent meristematic region. Also notice the degree of vascularization. Foliar traces appear at bottom of photograph. X21.—Fig. 6. External view of plant similar to that in Fig. 5. Note broad apical region with small enation appearing near the center. The bulges in the axils of the older leaves have given rise to a large number of irregularly placed leaves.

a plant early in this phase of regeneration is shown in Fig. 6. Especially striking are the axillary swellings and the large number of leaves which have arisen from these regions. Of note also is the fact that activity of the axillary meristem, though admittedly disturbed, could not have been inhibited very much since these leaves are of considerable size when compared with the leaves of the apical meristematic region.

Plants have been kept for more than four months under irradiation and the pattern has not changed materially. Even plants which had been removed from these conditions after a month of exposure have not regained their normal stature.

It appears that under conditions of chronic irradiation as applied in this experiment the apical meristem is initially inhibited. Lateral and axillary growth continues and may be even stimulated though polarity in the latter case appears disturbed since at first only a mass of tissue is formed. After some time leaves are formed by these axillary bulges and still later, approximately 30 days after initial exposure, the apical meristem resumes its activity producing leaves and leaf-like enations in no orderly fashion and distributed throughout the entire apical region. No organized shoot apex can be determined. It is of interest that no cell damage of any extent can be found; thus one can hardly speak of selection of radiation resistant cells and their progeny as being responsible for the regenerative growth. Rather, it seems that the cells have become adjusted to the treatment and though they cannot resume the original pattern of growth they continue to produce new organs in a more or less orderly fashion. The disturbance of the phyllotaxy may not be a direct result of irradiation. The possibility that the spatial pattern of the shoot apex was disturbed by the continued lateral growth during the initial inhibition of the apical meristem cannot be excluded. However, this does not explain why leaves are formed predominantly rather than shoots.

ACUTE IRRADIATION

Observation and Discussion: In the case of acute X irradiation another phenomenon is encountered. This is a striking differential elongation of internodes as shown in Plate 3, Fig. 7. The plant on the left is the control; the one in the center received 500 r and the one to the

Fig. 7. Kalanchoë "Brilliant Star". From left to right the plants received 0 r, 500 r and 2000 r acute X rays, respectively. Note in the 2000 r plant the much shortened internodes 4 and 5 and the considerably longer internode 6. The 500 r treated plant shows an intermediate pattern.

right received 2,000 r. While it may not be too obvious in the center plant, it is quite evident in the one on the right that internodes 4 and 5 have remained short and internode 6 has elongated much more than usual. These plants were irradiated when leaf 5 was approximately five mm long which means that leaf 6 would have been present only as a primordium if present at all. Fig. 8 represents graphically the pattern of growth of the individual internodes. It can be seen that internodes 1 and 2 which at time of irradiation were close to their final length are not affected. Internode 3 which was at its most active stage of growth at time of irradiation shows a lower final height in those plants which received 2,000 r but there seems to be no difference between the 500 r and the control curve. Internode 4 was in the process of being formed when the irradiation occurred and it shows essentially the same pattern as the previous internode but the difference between the 2,000 r curve on the one hand and

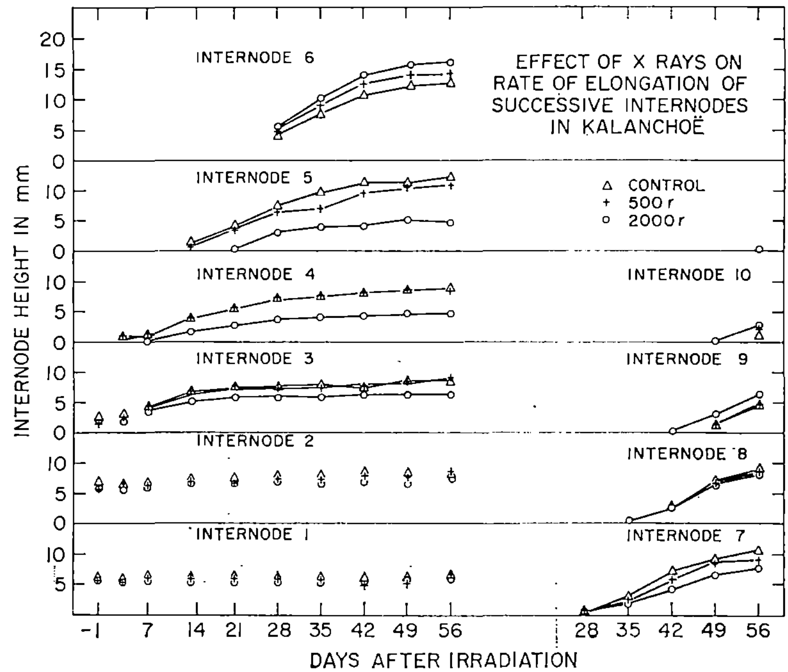

Fig. 8. Rate of elongation of successive internodes of Kalanchoë "Brilliant Star" after irradiation in late plastochron 5 and early plastochron 6. The early points are averages of at least 10 plants per treatment. Minimum number of plants per treatment was five.

the 500 r and control curve on the other has become accentuated. However, in the development of internode 5, which can be measured first about two weeks after the treatment, a dose effect becomes apparent. Not only is the 2,000 r group slower in development as indicated by the fact that it can be measured first only 3 weeks after irradiation and reaches only about half the length of the control but the 500 r group also grows more slowly and is consistently slightly shorter than the control. Internode 6 shows the striking reversal in pattern. All 3 groups can first be measured 4 weeks after irradiation, and there is no doubt that the internodes of plants receiving 2,000 r grow faster than the control group thus gaining considerably in length. The 500 r group assumes an intermediate pattern. Internode 7 reverts to the original pattern with the irradiated plants showing dose dependent degrees of inhibition. The patterns become obscured in the younger internodes because of the difference in time of initiation of internode elongation. There is a suggestion, however, that the rates of elongation remain the same in these internodes.

The time sequence of internode elongation is also reflected in the rate of leaf formation as shown in Fig. 9. The initial lag is quite striking in the 2,000 r group and it can be seen that growth resumes at 14 days after irradiation at an accelerated rate and continues for another 14 days. During this "spurt" more leaves are produced than in the controls over a similar period of time so that when the rates of leaf production become essentially the same in all three lines at 28 days after irradiation the 2,000 r group maintains this slight advantage in leaf number. Again the 500 r group presents an intermediate pattern.

It thus appears that acute irradiation temporarily stops shoot apex activity and also inhibits growth of those internodes which are in their maximum growth phase. The following internode grows

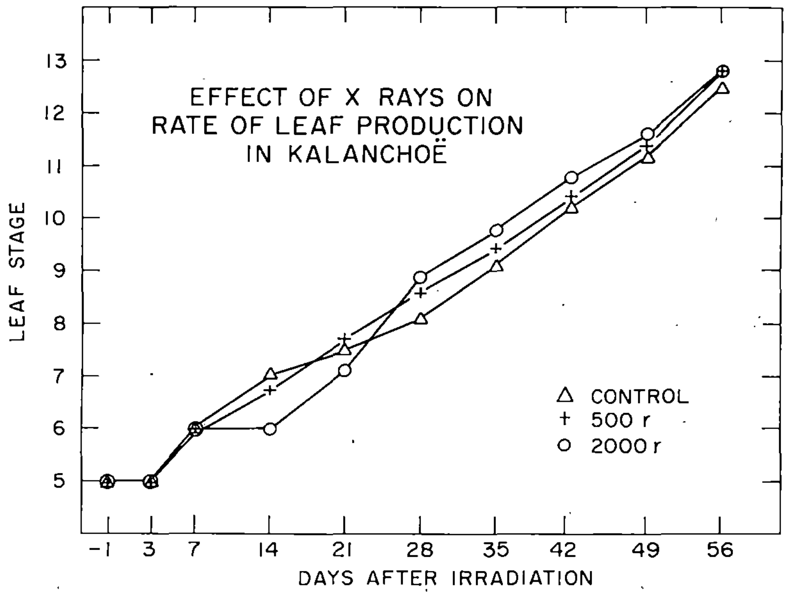

Fig. 9. Rate of leaf formation of Kalanchoë plants after irradiation. Note temporary inhibition of the plants which received 2000 r and their subsequent recovery. Same population as represented in Fig. 8.

beyond the extent of the controls. However, total height of the irradiated plants never equals that of the controls. The shoot apex after resumption of activity produces leaves at a higher rate than the controls for a long enough period of time so that the irradiated plants ultimately have more leaves than the controls at any point in time. Whether this effect is auxin mediated is currently under investigation.

Both stimulation and inhibition of growth as a result of radiation are well known (Sax 1955) though usually both phenomena do not occur in the same organ system. Johnson (1948) found that the irradiation of propagules of *Kalanchoë tubiflora* with 2500 r of X rays resulted in increases in number of plantlet-bearing leaves, number of plantlets and greater initial plant height. This species, however, is sufficiently different from K. "Brilliant Star" in morphology and apparently also radiosensitivity that a closer comparison is not warranted.

SUMMARY

In these experiments there are two different modes of application of ionizing irradiation and the results are quite dissimilar. In the case of chronic irradiation the terminal meristem apparently is inhibited for sufficient duration so that lateral meristematic activity becomes very pronounced and continues even after the cells of the apical meristem have recovered their ability to divide and form organs. In the case of acute irradiation if given in low enough dose the primary effect will be a temporary inhibition of the apical meristem and then the resumption of growth at an accelerated rate. This is also reflected in the growth of the internodes which are being formed at time of irradiation or immediately thereafter. Those internodes which are in the early stages of their grand period of growth at time of irradiation are much shorter at maturity than homologous non-irradiated internodes and the following internode is of considerably greater length. Cursory examination indicates that this is largely a matter of cell number rather than cell size.

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