# Studies on the Effects of Gamma Radiation on *Kalanchoe Pinnata* (Pers.), Kataka-taka (Tag.), Life Plant (Eng.)

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## ABSTRACT

*Somatic and genetic abnormalities were induced. The percentage of regeneration of irradiated leaves was reduced markedly at a dose of 2000 r and the reduction increased linearly with increased radiation dose. Plantlet growth decreased linearly with increased dose and was evident even seven months after irradiation in mature plants. The types of chlorophyll deficiency mutant plantlets observed were dark green, yellow-green, yellow and white. The greatest frequencies of chlorophyll mutations were recorded at moderate doses of 3000 r and 4000 r. The frequency of induced morphological abnormalities, i.e., the presence of abnormalities in leaves like abnormal serrations, cordate apices, lack of serrations and presence of twin shoots, increased linearly with increasing radiation dose.*

## INTRODUCTION

Interest in the induction of mutation in vegetatively propagated plants began with the discovery of efficient methods of detecting mutations and the discovery of effective mutagens. Work, however, was not as extensive as that done on seed-propagated plants. The latter has the advantage of gametophytic screening of induced genetic and chromosome lethalities.

Experiments on the induction of mutations in vegetatively propagated plants are faced with the difficulty of coming up with uniform results. Much of the difficulty lies in the different modes of reproduction, the genetical set-up of clonar varieties, the type of organ irradiated, the mode of growth, the stage of vegetative growth and the subsequent handling of the material. On the other hand, methods of inducing mutations in vegetatively propagated plants more or less differ from those used in seed propagated plants. Thus, induction of mutations in both kinds of species has the same theoretical foundations (Gaul 1964; Nybom and KKoch 1965).

The potential of the mutagenic effects of radiation on vegetatively propagated plants has been demonstrated in many agricultural crops such as potato (Van Harten et al. 1972), grape and peach (Lapins et al. 1969) and several ornamental plants like chrysanthemum (Bowens 1965), carnation (Buiatti and Ragazzini 1964) and gladiolus (Buiatti et al. 1965). The initial experiments done on the effects of radiation on *Kanlanchoe* were with the use of x-rays (Naylor 1931) on the *Bryophylum calycinum* which has the same regenerative ability as the *Kalanchoe pinnata.* These demonstrate that increasing the dose of X-ray radiation brought about a corresponding increase in regenerative ability.

Mutations in carnation after gamma irradiation were caused by alterations in a polygenic system. These mutations were propagated vegetatively up to the $vM_3$ generation. Ferweda (1965) experimented on potato using x-rays and EMS. He obtained mutations which could be perpetuated vegetatively. In one instance, the ivy-leaf mutant could be propagated even through seed. Van Harten et al. (1972) report that the ivy-leaf mutant is a dominant trait without pleiotropic effects.

The general effect of radiation is the inhibition of normal physiological processes such as normal growth, regeneration and fertility. However, several studies have demonstrated the stimulatory effects of radiation. Studies on the effects of $CO_2$ on gamma-irradiated plantlets of the *Kalanchoe* showed that irradiation had observable stimulatory effects on seedling height (Stein and Sparrow 1965). They attributed this increase to the unusual increase in the length of the first and second internodes of the seedlings. However, as the seedlings matured, there was a relative decrease in height which was attributed to the inability of the succeeding internodes to elongate. Similar findings on initial seedling height stimulation have been demonstrated in experiments on *Pinus rigida* (Mergen and Johnson 1964) and on the Helianthus (Skok et al. 1965). It has been pointed out that the cause of the initial stimulation is the presence of pile up precursors which were not utilized for the early stages of growth, but which later contributed to the unusual growth of the seedlings (Stein and Sparrow 1965).

## MATERIALS AND METHODS

The species used for this study was *Kalanchoe pinnata* (Pers.) which has several medicinal properties. It is locally called katakataka (Tagalog) or *siempre viva* (Spanish).

Juice from the leaves is used as an astringent, antiseptic, counterirritant against poisonous insect bites and medicine for earache and ophthalmia (Quisumbing 1951). Mixed with lard, the juice is used for diarrhea, cholera and phthisis.

Dalziel and Dymock (in Quisumbing 1951) state that the juice is used as a diuretic and as a cure for bilious diarrhea and lithiasis. Quisumbing further states that fresh leaves are pounded and applied to burns or as poultices on boils. Pounded leaves are used also as poultices on the soles of the feet to stop hemorrhages (Blanco 1878), as topicals in dislocations and callositis (Guerrero 1930). Mixed with salts, they are applied as a plaster to the abdomen to relieve enuresis (Sulit in Quisumbing 1951).

Friese (in Quisumbing 1951) states that the leaves are used as an emollient and refrigerant on swellings caused by neuralgia or toothaches. Rivera (1941) reports that the juice of the leaves is used in the tratment of acute nephritis.

Three hundred fifty leaves from 240-250 plants raised from one plant of *Kalanchoe pinnata* Pers. were selected based on age of the leaf, number of notches and leaf size. The leaves for treatment were simple leaves which were the sixth or seventh pairs of leaves of each plant.

After thouroughly washing the leaves with tap water and distilled water, the leaves were treated with gamma radiation in doses ranging from 1000 r to 6000 r. Each dose was administered to 50 leaves while a set of 50 non-irradiated leaves served as control. After irradiation, the leaves were rehydrated in distilled water for 30 min in a water bath with a constant temperature of

32°C. After rehydration, the leaves were set in pans containing washed sand and kept constantly moist with a thin layer of water over the sand surface. The leaves were sprouted in partial shade.

Thirty days after setting the leaves in the pans, they were scored for number of plants sprouting per leaf, plantlet height, number of leaves per plantlet and somatic mutations in the form of leaf abnormalities and chlorophyll mutations. The plantlets were detached from the leaves and transplanted in field plots according to a dose-to-row plan. The distance of planting was 30 cm between rows and 18 cm between plants.

Seven months after transplanting the VM (first irradiated vegetative generation) plants were scored for plant height and occurrence of induced leaf abnormalities. Fifty $VM_1$ leaves of each dose were grown in pans containing water and washed sand. Pans were checked continually to insure that the leaves were covered with water. After 30 days, the $VM_2$ leaves were scored for the number of plantlets per leaf, plantlet height, occurrence of somatic mutations in the form of leaf abnormalities and chlorophyll mutations.

## RESULTS AND OBSERVATIONS

**1. Percentage regeneration of plantlets.** In view of $VM_1$, the effects of gamma radiation on regeneration are shown in Table 1. When the regeneration, expressed as a percentage, is plotted as function of gamma radiation dose, a dose-effect relationship is observed. As radiation dose was increased from 1000 r, the number of plantlets regenerated per leaf decreased. Compared with the control, relatively fewer plantlets grew from leaves irradiated with doses ranging from 2000 r (70.50%) to 6000 r (38.30%).

There was a slight increase in the number of plantlets at 1000 r (102.87%). However, when higher doses were applied, a dose-effect relationship was seen. As the radiation dose increased, there was a decrease in the number of plantlets regenerated per leaf.

In the $VM_2$, as shown in Table 2, there was a slight stimulation in the number of plantlets that germinated at the lowest dose of radiation (100 r). Just like the $VM_1$, when higher doses were applied, there was a decrease in the number of plantlets as compared with the control.

**2. Height of plantlets.** Table 2 shows the mean heights of plantlets measured 30 days and 210 days after treatment. Plantlet height reduction was obtained which increased more or less with dose.

Data show that 30 days after irradiation, there was a slight increase in plantlet height at 1000 r (101.98%) as compared with the control. A gradual decrease in plantlet height was seen at higher doses of radiation. After 210 days, there was an obvious dose-effect relationship seen at all doses.

**3. Chlorophyll mutations in the $VM_2$.** It is evident from the data (Table 4) that the highest mutation frequency was obtained at moderate doses of 4000 r (12.60%). The frequency dropped abruptly at 5000 r (7.56%) and finally to 6000 r (5.97%), the lowest frequency.

The chlorophyll mutations spectrum (Table 5) shows the different types of chlorophyll mutants observed: viridis (deep green), chlorina (yellow-green), xantha (yellow) and albina (white). The frequency of each type of mutation is expressed as a percentage of the total mutant types. The highest frequency was that of the chlorina type at 3000 r (68.63%). Xantha were oberved only at 3000 r (5.2%) and albina were observed only at 4000 r (2.33%). In all doses, the chlorina mutants were the most frequent while xantha and albina were very rare.

**4. Morphological abnormalities.** The different kinds of morphological abnormalities induced by gamma radiation were leaf abnormalities with rounded apices, lack of normal serrations and cordate leaves (Table 6).

**5. Frequency or morphologial abnormalities in the $VM_2$.** Table 7 shows that the $VM_1$ scored after 90 days increased in the number of leaf abnormalities with increased radiation dose. After 210 days, there was also seen an increasing frequency of abnormalities as the dose increased to 4000 r. There was a slight decrease in the frequency from 4000 r (36.54%) to 5000 r (29.41%). The highest frequency of abnormalities was obtained at the highest dose of 6000 r (39.13%).

For the $VM_2$ generation, the data show an obvious increase in the frequency of abnormalities as the dose of radiation increased. The lowest percentage was obtained at the lowest dose (616%) and the highest percentage at the highest dose (1766.18%).

## DISCUSSION

From the foregoing results, it is evident that gamma rays affected the generative capacity of leaves, plantlet height, shape and form of leaves in *Kalanchoe pinnata* Pers. These effects are useful biological indices of the radiosensitivity of a species. Several mutation experiments have shown that results obtained may be specific for each mutagenic agent and that response varies with different biological materials, treatment conditions and post treatment techniques. Mutagenic efficiency is known also to be influenced by several biological factors such as nuclear volume, cellular stages, variety of species, the age or stage of development and the radiosensitivity of each plant material.

Leaves of *Kalanchoe pinnata* Pers. that were exposed to gamma radiation showed a significant decrease in the number of plantlets regenerated per leaf (Table 1), a distinctly notable decrease in seedling height as radiation dose increased, the appearance of chlorophyll mutations whose frequecy increased at moderate doses but which decreased as higher doses were applied, and the appearance of an increasing number of leaf abnormalities with increasing doses.

Differences in opinion as to the number of meristematic cells that give rise to young plantlets in vegetatively propagated plants make it difficult to study the effect of radiation on the regeneration of plantlets. However, the fundamental role of meristematic cells in morphogenesis and their sensitivity to radiation are well recognized. Meristematic cells are more susceptible to radiation damage than resting cells (Gunckel 1957). Gunckel further states that both chromosomal damage and mitotic inhibition of irradiated cells are the effects of irradiation. Evans (1965) pointed out the effects of radiation on meristematic cells such as mitotic cycle delay, formation of chromosomal aberrations and loss of proliferative capacity due to either premature differentiation or cell death.

It has been observed that there was a reduction in the number of plants that regenerated per leaf as radiation dose increased. Evidently, this reduction cannot be ascribed to mitotic cycle delay because no new growth occurred. Neither can it be attributed to lethal chromosomal aberrations, as limitations of the study did not include cytological examinations. However, the reduction may be explained by the loss of proliferation capacity as a result of either premature differentiation or cell death.

Using leaf development as an index of the biological effects of gamma rays, many abnormalities of leaf shape were observed. More explicitly, irradiated *Kalanchoe pinnata* leaves yielded plants with some leaves that had rounded apices, lacked normal serrations or were heart-shaped. All the plants that grew from leaves treated with different doses of gamma rays produced leaves which lacked normal serrations. Non-serrated leaves appeared in all the groups dosed with gamma rays.

Leaves with non-serrated margins were also observed in the control plants. While less non-serrated leaves were obtained from the unirradiated plants than from the irradiated plants, this indicates that spontaneous mutations were produced probably under normal growth conditions. It has been suggested that the failure of leaf blades to develop properly is to be attributed to high concentrations of phytohormones, chromosome fragmentation and normal deficiencies (Gunckel 1957). Spontaneous mutations are caused by point mutations (Gaul 1964; Konzak et al. 1965).

Abnormal leaf shapes were observed as early as the six-leaf stage (85 days after parent leaves received gamma irradiation). Irregularities in leaf shape were observed to increase in number in the first four leaf pairs of the plants, but decreased unmistakably with the appearance of new leaves as the plants grew older (up to seven months). This may be explained as being due to the capacity of the plants to recover from the effects of radiation and the inherent ability of the plant to repair damaged tissue.

The presence of heart-shaped leaves (Fig. 3) could be explained by the death of cells in the center of the meristematic regions which have specific influences on the development of leaves and leaf shape. Thus, leaf aberrations may be largely due to the application of lethal doses of radiation affecting early stages of embryonal development, thereby resulting in leaf abnormalities. Assuming that physiological activity has started in the meristematic regions toward the formation of new plants, gamma radiation may have adversely affected certain embryonal mechanisms which resulted in the non-development of leaf apices. Thus, the appearance of bifurcated or heart-shaped leaves in the progenies.

Damage to the tissues, organs and the whole plant as discussed, represent the morphological and physiological effects of irradiation. On the other hand, the genetic effects of radiation are best exemplified by the presence of chlorophyll mutations (Table 3).

Several types of chlorophyll mutations were observed: viridis (dark green), chlorina (yellow green), xantha (yellow), and albina (white). It was observed that the incidence of xantha and albina were proportionately much less as compared with the other types of chlorophyll mutations.

These studies demonstrate that one of the biological effects of ionizing radiation is the induction of chlorophyll mutations. These mutations are believed to be caused by changes in the chromosome structure -- either a break or a deletion. This would mean changes in the nucleotide sequence which would mean a change in the information coded in the DNA. Sylenga (1964) attributes dominant chlorophyll mutations to chromosomal aberrations of the 2-break type (2 hit events). Changes in the color of *Streptocarpus* (Strickberger 1969) are due to biochemical effects which can be traced to separate genes. These genes appear to produce their effects by the addition or subtraction of hydroxyl (OH) or methoxyl (O-$CH_3$) units to the sugar of the DNA molecule. Thus, any change in the genetic material could bring about changes in the production, function and specificity of an enzyme: in this case, the production of normal pigmentation. The degree to which plants respond to increasing radiation dose indicates the extent to which injury influences the production of chlorophyll pigments. Chlorophyll mutations may be attributed to proplastid damage or the deletion of one or more genes (Love 1969).

Ionizing radiation affects the genetic make-up by transferring its energy to atoms or molecules present in or near the cell nucleus. Radiation energy causes molecular breakage which provides substrates for chemical reactions in the cell. Thus, in the case of chlorophyll mutations, the DNA molecule is affected directly or indirectly by ionizing radiation.

Any change in the molecular structure of the genes would change the information coded in the DNA. A gene, which is a segment of a DNA molecule, is responsible for the synthesis of an enzyme. Any change in the DNA molecule would bring about a change in the gene, thereby inhibiting the normal synthesis of the enzyme.

Concomitant to the different stages of development of normal chloroplasts is pigment formation. From a colorless granule, yellow pigments are initially formed, followed by green pigments. The different genes controlling pigment formation and the development of chloroplasts act in series, each gene carrying on where the previous gene left off. The effect of mutation on the different genes becomes evident in the inhibition of succeeding stages, resulting in the formation of chlorophyll mutations.

In the present study, the life span of the albina mutants was very short. This can perhaps be attributed to extensive radiation damage, which gave no chance for recovery. Studies have shown evidence of recovery and repair of induced physiological damage and of a greater part of genetic damage. These can explain the disappearance of chlorophyll mutations in the *Kalanchoe pinnata* after 30 days of growth.

Chlorophyll mutations obtained in the $VM_2$ generation show that the highest frequecy of abnormalities was obtained at 4000 r. A decrease in frequency of mutants was quite evident at increased doses of radiation. This decrease could be attributed to repair and survival . Of the chlorina mutants obtained at the $VM_2$, the most abundant were the chlorina mutants in plantlets irradiated at 3000 r. Chlorina and viridis mutants were obtained in all doses. Only one xantha and one albina were obtained at 3000 r and 4000 r, respectively.

These results indicate that at higher doses, the progress of the DNA-repairing phenomenon could be hindered possibly by the inactivation of the repair system. Increase in radiation increases the number of deletions or breaks. As a consequence, there is a change in the nucleotide sequence which brings about a change in the DNA information. These changes in the DNA code are carried over the $VM_2$ generation.

## SUMMARY AND CONCLUSIONS

Leaves of *Kalanchoe pinnata* were exposed to gamma radiation with doses starting from 1000 r. After irradiation, they were rehydrated and sprouted in pans containing water and sand. After 30 days, plantlets which regenerated at the margins of the leaves were scored for height, number of plantlets regenerated per leaf and morphological and chlorophyll mutations. Results showed that irradiation caused biological damage shown in the reduction of the number of plants regenerated per leaf, reduction in height and occurrence of leaf abnormalities and chlorophyll mutations.

Gamma irradiation produced both somatic and chlorohpyll mutations in *Kalanchoe pinnata.* The occurrence of morphological abnormalities was dependent on radiation dose: the higher the dose, the greater the number of leaf abnormalities.

Inhibitory effects of irradiation are indicated in the decrease in the number of plantlets regenerated per leaf and the decrease in plantlet height.

Somatic mutations included twin shoots and leaf abnormalities like lack of serrations and rounded or heart-shaped apices. These are useful indices of mutagenicity. The application of higher doses of radiation increased the frequency of leaf morphological abnormalities. However, it did not cause a proportional increase in chlorophyll mutations. Chlorophyll mutation frequency was greatest at moderate doses of 4000 r.

Occurrences of chlorophyll mutant plantlets such as viridis, chlorina, xantha and albina are good indices of genetic mutations. Chlorina mutants were the most frequent, while albina and xantha types were rare.

Gamma irradiation produced both morphological and genetic effects in *Kalanchoe pinnata*. In this study, the frequency of chlorophyll mutations was not proportional to dose of radiation. However, morphological abnormalities were shown to increase proportionately with increasing radiation doses. Radiation, as evidenced by the occurrence of morphological abnormalities, speeds up the occurrence of variations already found in nature.

Table 1. Percentage regeneration of plantlets of *Kalanchoe pinnata* leaves after gamma irradiation

| Gamma ray dose | Number of plantlets per Range | $VM_1$ Leaf ± Mean | Percentage Regeneration |
|---|---|---|---|
| 0 | 2 - 11 | 5.56 ± 3.04 | 100.00 |
| 1000 r | 2 - 11 | 5.72 ± 2.07 | 102.97 |
| 2000 r | 1 - 9 | 3.92 ± 1.53 | 70.50 |
| 3000 r | 1 - 7 | 3.87 ± 1.85 | 69.60 |
| 4000 r | 1 - 7 | 3.73 ± 1.64 | 67.08 |
| 5000 r | 1 - 6 | 3.00 ± 1.43 | 53.95* |
| 6000 r | 1 - 4 | 2.13 ± 1.002 | 38.30* |

* Significant at the 5% level

Table 2. Mean height of $VM_1$ plantlets of *Kalanchoe pinnata* after gamma irradiation

| Gamma ray dose | Range (cm) | Mean (cm) | Percentage of control |
|---|---|---|---|
| 30 days after irradiation | | | |
| 0 | 3-37 | 17 71 ± .921 | 100.00 |
| 1000 r | 8-28 | 18.06 ± .495 | 101.98 |
| 2000 r | 9-34 | 17.67 ± .1140 | 99.77 |
| 3000 r | 7-32 | 17.36 ± .848 | 98.02 |
| 4000 r | 8-30 | 15.67 ± .789 | 88.48 |
| 5000 r | 5-40 | 14.86 ± .872 | 83.91 |
| 6000 r | 6-20 | 7.33 ± .440 | 41.49* |
| 210 days after irradiation | | | |
| 0 | 100-460 | 224.41 ± 72.09 | 100.00 |
| 1000 r | 60-420 | 195.85 ± 28.04 | 87.27 |
| 2000 r | 70-404 | 194.39 ± 25.55 | 86.62 |
| 3000 r | 32-398 | 183.57 ± 44.59 | 81.80 |
| 4000 r | 65-285 | 178.46 ± 74.25 | 79.52 |
| 5000 r | 80-275 | 158.69 ± 43.52 | 70.71 |
| 6000 r | 54-235 | 120.88 ± 48.68 | 53.87* |

* Significant at the 5% level

Table 3. Percentage regeneration of $VM_2$ plantlets in *Kalanchoe pinnata* after gamma irradiation

| Gamma ray dose | Range | Mean number of plantlets per leaf | Percentage regeneration |
|---|---|---|---|
| 0 | 4-16 | 9.60 ± 1.97 | 100.00 |
| 1000 r | 4-18 | 10.50 ± 2.75 | 109.37 |
| 2000 r | 3-17 | 9.20 ± 3.63 | 95.83 |
| 3000 r | 3-16 | 8.08 ± 3.93 | 84.17 |
| 4000 r | 3-15 | 7.92 ± 3.76 | 82.50 |
| 5000 r | 3-16 | 7.52 ± 3.56 | 78.33 |
| 6000 r | 4-16 | 7.32 ± 3.06 | 76.25 |

**Table 4.** **Frequency of chlorophyll mutations in $VM_2$ plantlets of *Kalanchoe pinnata***

| Gamma ray dose | Total number of plantlets scored | Number of chlorophyll mutants | Frequency % |
|---|---|---|---|
| 0 | 473 | 0 | 0 |
| 1000 r | 490 | 46 | 9.39 |
| 2000 r | 505 | 49 | 9.70 |
| 3000 r | 436 | 51 | 11.70 |
| 4000 r | 341 | 43 | 12.60 |
| 5000 r | 397 | 30 | 7.56 |
| 6000 r | 385 | 23 | 5.97 |

**Table 5.** **Chlorophyll mutation spectrum in $VM_2$ plantlets of *Kalanchoe pinnata* induced by gamma irradiation**

| Gamma ray dose | Total number of mutants | V % | Ch % | X % | A % |
|---|---|---|---|---|---|
| 0 | | | | | |
| 1000 r | 46 | 39.13 | 60.87 | | |
| 2000 r | 49 | 46.94 | 53.06 | | |
| 3000 r | 51 | 25.49 | 68.63 | 5.88 | |
| 4000 r | 43 | 32.56 | 65.12 | | 2.33 |
| 5000 r | 30 | 40.00 | 60.00 | | |
| 6000 r | 23 | 34.00 | 65.22 | | |

V - viridis
Ch - chlorina
X - xantha
A - albina

**Table 6. Morphological abnormalities induced by gamma irradiation of the *Kalanchoe pinnata***

| Plant character | Char. of variant | Irradiation dosage |
|---|---|---|
| 1. Serrated leaves | Leaves which lack serrations | All doses including control |
| 2. Pointed apex | Rounded leaf apices, cordate apices sometimes without normal serrations | 1000 r - 6000 r |
| 3. Single primary shoot | Plants with multiple primary shoots usually 2 | All doses except at 2000 r |

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