การใช้รังสีแปรกรรมพันธุ์ในต้นฆ้องสามย่าน (Kalanchoe laciniata)

Radiation-Induced Somatic Mutations in Kalanchoe (Kalanchoe laciniata.)

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ABSTRACT

Five doses of gamma radiation, i.e. 1,000 r, 2,000 r, 3,000 r, 4,000 r and 5,000 r at a dose rate of 72 r/min. were used to produce somatic mutation in *Kalanchoe* through leaf petiole cuttings.

Many different mutants were produced from irradiated petioles. These mutants mostly exhibited changes in leaf structures eg. leaf margins, leaf shapes and colour. Out of various mutants, the one obtained from the irradiation of 4,000 r has a striking appearance. It is the dwarf plant with alteration in colour from normal green to purplish colour. From the experiment, it can show beyond doubt the success of induction of somatic mutation in vegetatively propagated plant.

Production of radiation - induced somatic mutations; particularly those affecting fruit, flower or other general morphology, in vegetatively propagated materials is a present being investigated on a rather wide scale in many countries (2,5,6,7). The various types of diaspores used in vegetative propagation also have been exposed to radiation, including scions, buds, cuttings, bulbs, tubers, leaf-petiole cuttings, rhizomes, etc. The most common doses that have been used in somatic treatment lie between 2,000 and 4,000 r units of x-rays or gamma-rays (6). Most of the primary mutants from irradiated vegetative tissues are chimaeric except from leaf -petiole cutting. Sparrow et. al. (8) reported that a new plantlet (or shoot) can originate from a single epidermal cell of the petiole.

In 1963 Stein and Sparrow (9) studied the effect of chronic gamma irradiation of the growth of a common horticultural *Kalanchoe* resulted in many forms of malformed leaf structures. Kalanchoe plant can be propagated by leaf-petiole cutting.

Irradiation petiole of this plant may induce some somatic mutation or changes on leaf or plant morphology.

The study undertaken was aimed to investigate the possibility of inducing somatic changes in the developing Kalanchoe plant by irradiating leaf-petiole cuttings with various doses of gamma rays.

Materials and Methods

Kalanchoe laciniata is a succulent plant of the family crassulaceae. The leaf has short petiole with ovate leaf structure, crenate - dentate margin. The flowering is yellow, nearly 1/2 inch across (1).

The procedure used in the experiments was to carefully remove fully grown leaves 8 to 10 leaves per plant from healthy plants. The leaves were immediately taken to the irradiation room in the radiation greenhouse and irradiation commenced without delay. Leaves were arranged so that the bases of the petioles received radiation doses as required. Irradiation treatments consisted of one control and 5 radiation levels, i.e., 1,000 r, 2,000 r, 3,000 r, 4,000 r and 5,000 r at a dose rate of 72 r per minute, 20 leaves were used for each treatment (Table 1).

Table 1 Number and percentage of survived petioles of *Kalanchoe* observed in control and irradiated treatments with different gamma doses.

Treatments	Dose r	Dose rate r/min	Number of leaf cutting	No.of survived leaves	Percentage of shooting petiole
1	Control	-	20	13	65
2	1,000	72	20	8	40
3	2,000	72	20	7	35
4	3,000	72	20	7	35
5	4,000	72	20	10	50
6	5,000	72	20	8	40

After irradiation the leaves were planted in moist medium (mixture of one part of sand and one part of ash) with only the portions of petioles being inserted in the medium.

Results

The earliest indication of growth activities is the formation of callus tissue over the cut surface of petioles. There are no plantlets arising above the irradiated portion of the petioles so that all new plants arose from irradiated tissue. After young plantlets were formed in medium, they were transfered to 6 inch clay pot, which were grown to about six weeks and scored as either normal or mutant.

Percentage of the leaf cutting success, shooting time, and number of shoots or plantlets appearing from the leaf cutting was observed regularly (Table 1 and 2).

Table 2 Age of survived leaves from planting to the formation of plantlets per leaf.

Treatments	No. of survived plantlets	Average days taken to emergence	Average no. of plantlets per leaf
Control	13	28.46	1
1,000 r	8	41.71	1
2,000 r	9	49.43	1.29
3,000 r	7	53.29	1
4,000 r	13	84.60	1.30
5,000 r	11	54.38	1.38

Many different mutant types and temporary aberrant growth were produced from irradiated petioles.

Percentage of shooting petioles in control leaf cutting was 65 per cent in treated leaf showing lower 40, 35, 35, 50 and 40 per cent in 1,000, 2,000 3,000, 4,000 and 5,000 r respectively (Table 1).

Average duration of time taken from cutting to appearing of shoot from petiole were 28.46 days in control, and 41.71, 49.43, 53.23, 84.60 and 54.38 days in 1,000, 2,000, 3,000, 4,000 and 5,000 r respectively. Most of the leaf cutting yielded one young plantlet per petiole particularly in control and treatment 1,000, 2,000 and 3,000 r (Table 2).

Mutant and aberrant growth.

Some minor aberrant growth has been observed in only one leaf of the young plant from control petiole while the other appearing normal.

A dose of 1,000 r caused some inhibition or retardation of shooting but occurrence of mutation or other changes can not be classified.

One plantlet grown from a petiole given 2,000 r has asymmetric development of leaf blade with entire margin. Mutant plants were observed from another petiole of this treatment which gives three plantlets all showing changes in leaf form, from ovate leaf to nearly round leaf (Fig.1).

A petiole treated with 3,000 r yielded mutant plantlet with the leaf blade of entire margin (Fig 1).

The action of 4,000r of gamma rays on petiole of *Kalanchoe* is capable of producing great changes in leaf character in respect to form, color, and structure (Fig 2). One mutant from this treatment is the dwarf plants with purplish color and curly leaves. The phenotype is completely distinct from the normal one. The mutant can be maintained through vegetative propagation. These dwarf mutant plants have never produced flower since they were formed. Other mutant change is the formation of slightly creanate leaf as compare to control plants (Fig. 2).

Most of the plantlets grown from petioles given 5,000 r show aberrant growth such as variegated leaf, deformed leaf, asymmetrical development. All these aberrants can be distinguished from the normal plants. Four plantlets from four petioles appeared with such aberrant growths. Only one mutant can be maintained from this treatment, the mutant having different leaf shape as seen in figure 3.

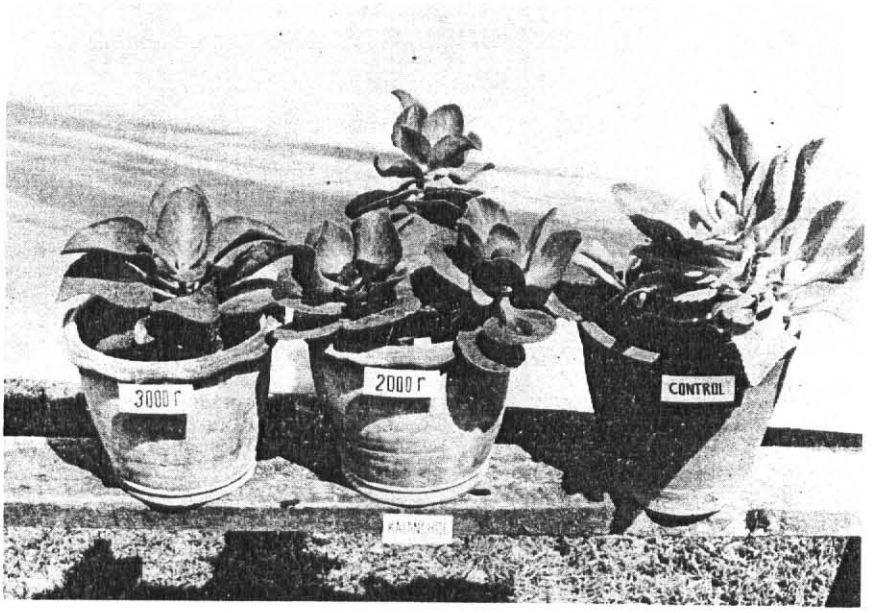

Fig. 1. Mutant plants with different leaf shapes resulting from treatments of 2,000 r and 3,000 r.

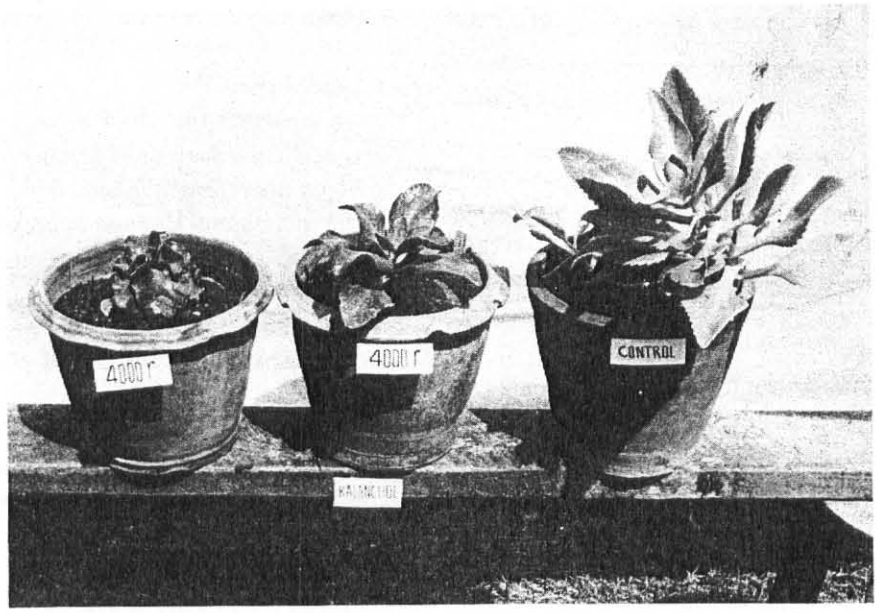

Fig. 2 Gamma ray-induced mutation in Kalanchoe. Two mutants obtained from 4,000 r. treatment, one with sligthly creanate leaves and the other at left, a dwarf plant showing great changes in shape and structure of leaves.

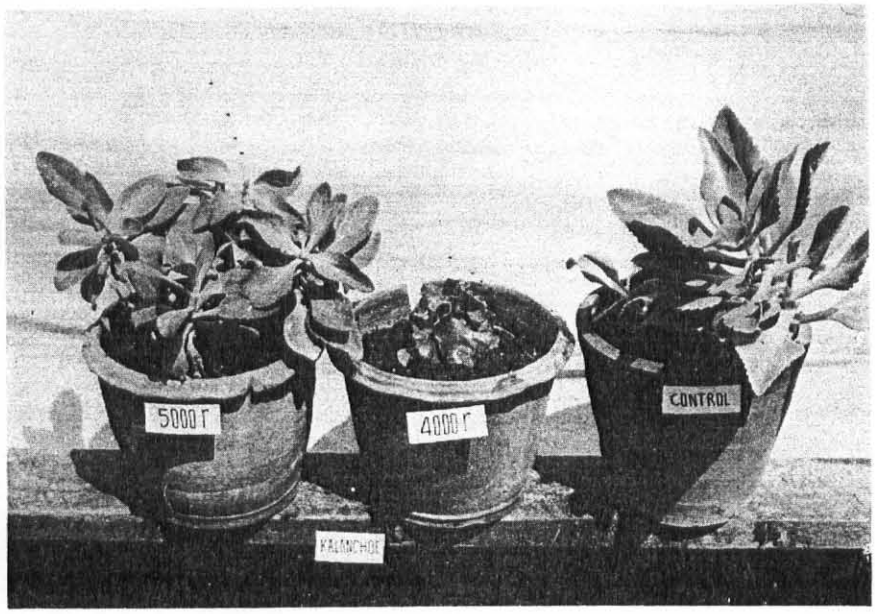

Fig. 3 Control plant compares with leaf mutant plants from the irradiation of 4,000 r. and 5,000 r.. Notice differences between these mutants.

Discussion

The experimental results show that the doses of 1,000 r to 5,000 r can cause inhibition or retardation of shooting percentage. Higher dosage in the vicinity of 4,000 r and 5,000 r produced more changes in leaf structure and delay of shoot development. It has been found that changes in enzyme activity and variety of physiological or metabolic disturbance occur after irradiation of plants (4) with retardation of cell differentiation may lead to inhibition of shooting percentage and development.

Number of new plantlets from each petiole decrease after irradiation with higher doses. This may be the effect of radiation depressing auxin levels. Skoog (cited from Gunckel and Sparrow, 1954) states that the development of lateral buds following irradiation is correlated with the destruction of auxin in the plant and is not the result of direct bud stimulation by X -rays. In 1953 Gunckel and Sparrow (3) also found that aberrant multiple shoots can be induced in *Tradescantia.*

Gunckel and Sparrow (4) summarized the facts that most plants produce leaf anomalies following suitable doses of ionizing radiation. The leaf anomalies include dwarfing, thickening, roughened, or abnormal curvatures of the blade, curling of leaf margins, distorted venation, fusions, cupshaped or tubular leaves, color changes and premature abscission. Possible causes of certain of these anomalies are associated with cell proliferation, cell enlargement, and some pulling apart of cells. In our experiment, some aberrant growth on leaf has also been found in plantlets from irradiated petiole with high doses.

Several of induced mutant forms can be maintained by vegetative propagation. In Saintpaulia, Sparrow and his co-worker (8) irradiate the leaf-petiole with 2,000 and 3,000 r X-rays and of 154 cases, 14.2 per cent, mutated plants were recorded respectively. A feature of special interest concerning these mutants is that they are mostly homogeneous, i.e., not chimaeric. This is to be expected as the new plants are derived from single cells of the primary, irradiated leaf petiole.

Mutant types which were obtained in irradiated *Kalanchoe* may represent a change in a single gene locus while the other may represent a structural chromosome change. The latter could be a deletion, duplication, translocation, or some other types of rearrangement. The exact nature of mutation has not been known. This requires further investigation by cytological analysis.

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