

CHAPTER - II

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EFFECT OF  $\gamma$ -IRRADIATION, A PHYSICAL MUTAGEN ON  
Crotalaria juncea Linn.  
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#### A. Introduction :

Induction of mutations using physical agents as  $\gamma$ -irradiation is a potent method of creating new genetic variability which enhances the scope of selection. These agents bring about breakage and point mutations in DNA no matter whether the plant organs are storage type, as seeds with low moisture content, or tissues with high moisture content. Such changes induced are readily visible in the seedlings when seeds germinate. If the dosage is too high i.e. lethal the seeds die, sublethal doses never the less, bring about number of phenotypic changes manifested as changes in morphology, physiology etc. in the  $M_1$  generation itself. Since chloroplast and mitochondria are also known to have DNA, such changes are also possible at their functional level.

Not all types of seeds respond in similar way. Depending upon their nature of storage material (oil, protein, starch), responses vary. The type of mutation brought by any mutagenic agent with respect to physical or chemical nature and the mechanism of action of the agent and the type of modification brought by a physical agent may be different from that of a chemical agent. So the attempt is made to study the nature of morphological and physiological changes brought by  $\gamma$ -irradiation a physical mutagen in Crotalaria juncea L.

## B. Material and Methods :

### 1. Morphological parameters :

#### (a) Germination and Survival :

Seeds of Crotalaria juncea were collected from the Agricultural College, Kolhapur for the study of the effect of  $\gamma$ -radiation on some morphological and physiological parameters.

Five lots of about 100 g seeds were  $\gamma$ -irradiated with 10, 20, 30 and 40 KR doses at Bhabha Atomic Research Centre, Bombay. Prior to irradiation moisture percentage of the seeds was determined by loss in weight method.

One hundred seeds of each of the treatment were sown in different pots for further investigation of seedlings and 100 seeds of each treatment were kept for germination in germination papers. Control was also sown and kept for germination. The rate of germination was scored based on 72 h of germination, and survival percentage was calculated after 15 days.

The methods followed, for the study of different morphological and physiological parameters have been described already (in chapter I) while studying the effect of DES on different parameters in C. juncea L.

### C. Results.

#### 1. Morphological parameters :

##### (a) Germination and Survival :

The moisture percentage of the seeds of Crotalaria juncea subjected to  $\gamma$ -irradiation is determined and it was found to be 6.483.

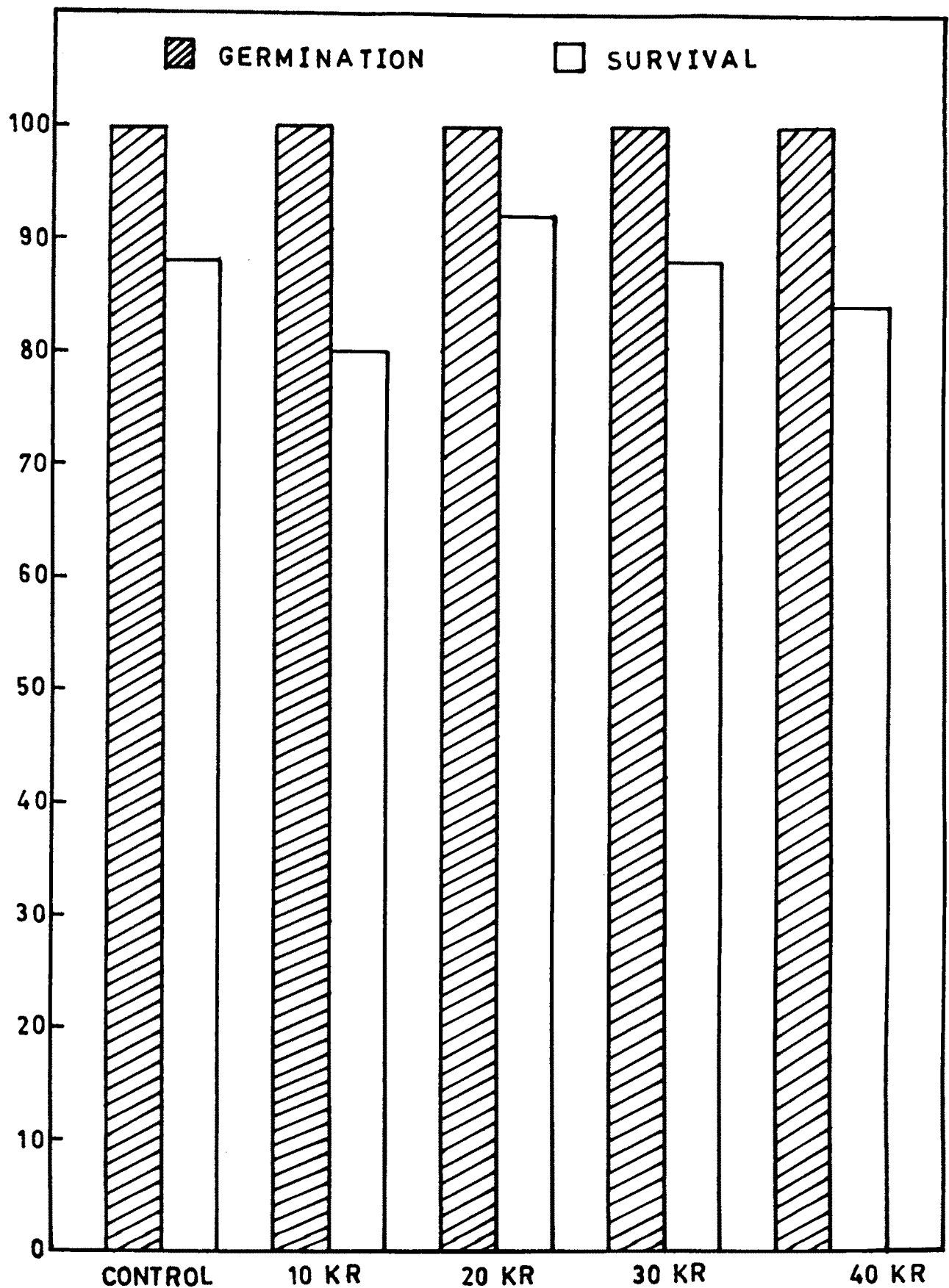
Irradiation effect on the rate of germination, overall germination percentage, as observed upto 72 h. as well as survival percentage after 15 days in  $M_1$  generation of C. juncea is depicted in Table 8 and Figs. 16 and 17.

Unlike that in DES treated seeds, germination in the irradiated seeds started on the same day as that of control. In other words no delay in the germination could be noticed. On the contrary germination is (positively) stimulated in  $\gamma$ -irradiated seeds over that of control (Fig. II). At 40 KR which is the highest dose chosen in the present investigation, the total percentage of germination is almost equal to control. In other words the dosage of irradiation required to reach LD 50 in this species of Crotalaria appears to be much higher.

Like germination, the survival percentage is not significantly affected by irradiation, conferring that

Table 8 : EFFECT OF  $\gamma$ -IRRADIATION ON SEED GERMINATION  
AND SURVIVAL OF Crotalaria juncea  
(M<sub>1</sub> generation).

Treatment	Hours after Germination				Percent age of germina tion.	Percent age of survival after 15 days.
	12	24	48	72		
Control	14	1000	100	100	100	88
10 KR	12	98	100	100	100	80
20 KR	12	96	100	100	100	92
30 KR	13	99	100	100	100	88
40 KR	13	100	100	100	100	84



G. 16 : EFFECT OF  $\gamma$ -RADIATION ON GERMINATION AND SURVIVAL PERCENTAGE OF Crotalaria Juncea L.-(M<sub>1</sub> GEN.)

the doses chosen are too low. Unlike in DLS treated, the 20 KR treated plants which show more vigour, the survival percentage is higher compared to others.

(b) Morphological peculiarities :

The seedlings obtained after seed treatment with various doses of  $\gamma$ -irradiation had their cotyledons with malformed shapes. In 10 KR certain seedlings observed with slightly crumpled cotyledons, some with one cotyledon very much reduced <sup>in size than the</sup> other normal. In 20 KR also such type of malformed cotyledons have been obtained. In 30 and 40 KR irregularities in cotyledon shapes has been increased. Crumpling of the cotyledon, mal formation of one of them or both, tricotyledonary nature and one seedling with two radicles (Fig.18) in 40 KR, are some of the significant peculiarities of plants obtained after irradiation.

Radiation has drastically affected the size and shape of the leaves. At the seedling level the marginal meristem of the leaves was affected, the leaves become distorted in shape. Some of the peculiar distortions found after 20 KR and 40 KR treatment respectively are shown in Figs. 19, 20 and Fig. 21 respectively. Sectorial discolorations in the leaves of C. juncea are observed from 20 KR onwards. The area discoloured being directly proportional to the level of the dose. Some of the leaves

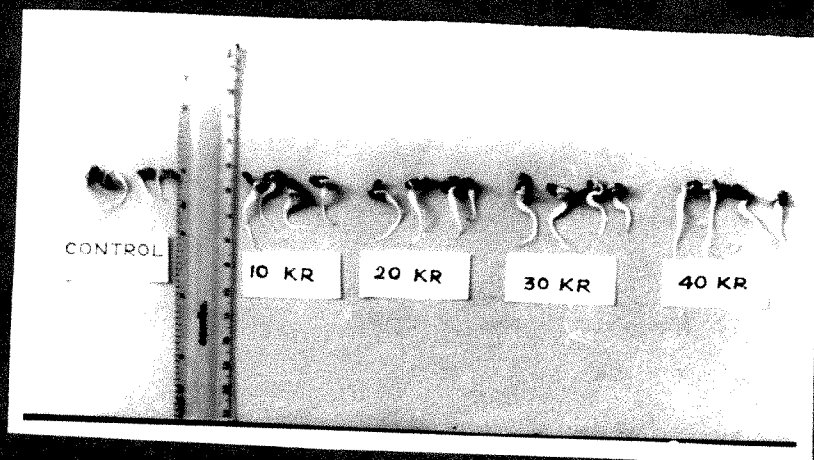


Fig . 17 .

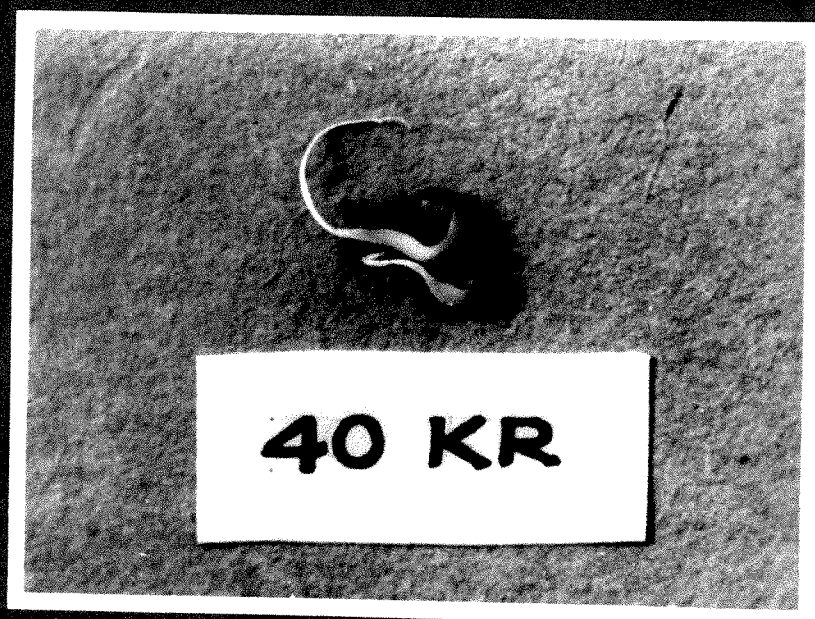


Fig . 18 .



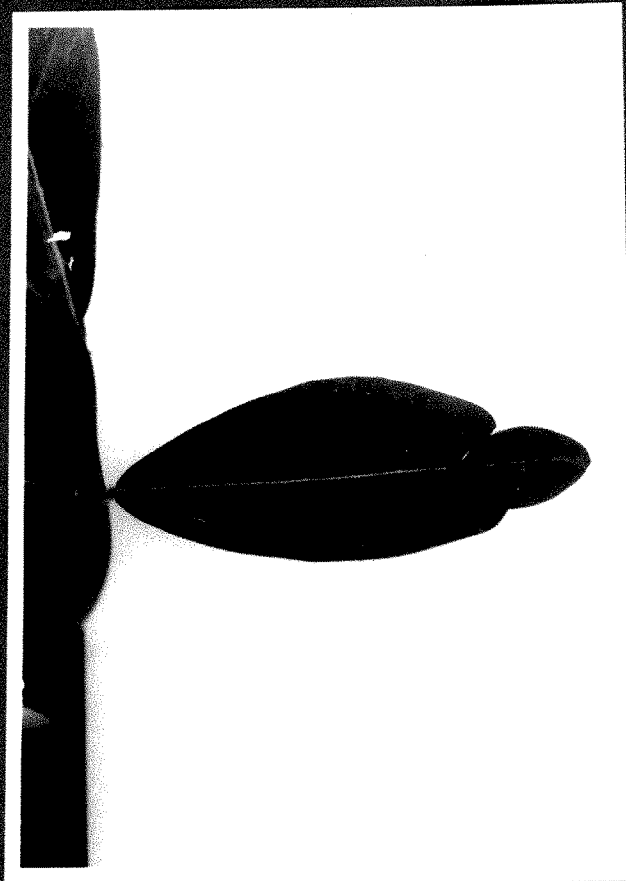


Fig. 19.

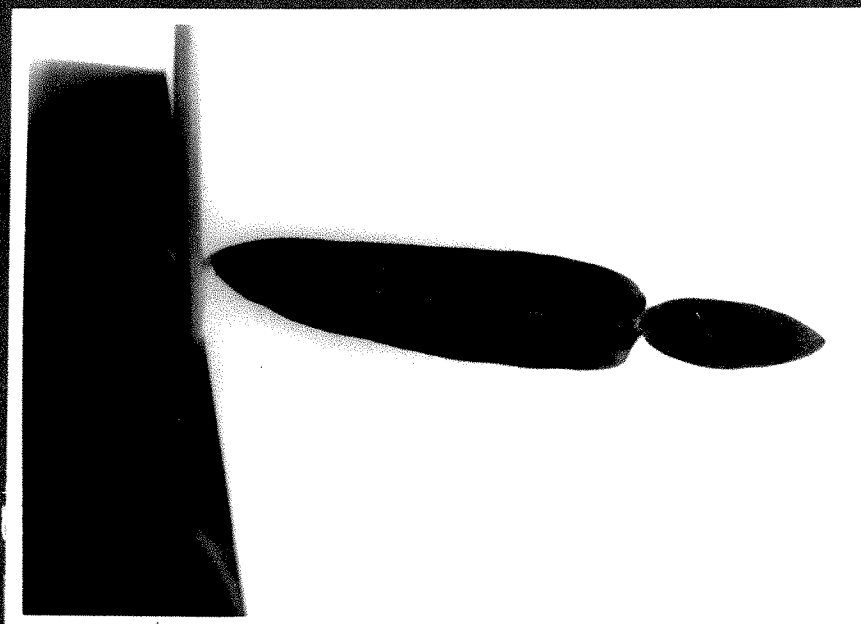


Fig. 20.



Fig. 21.

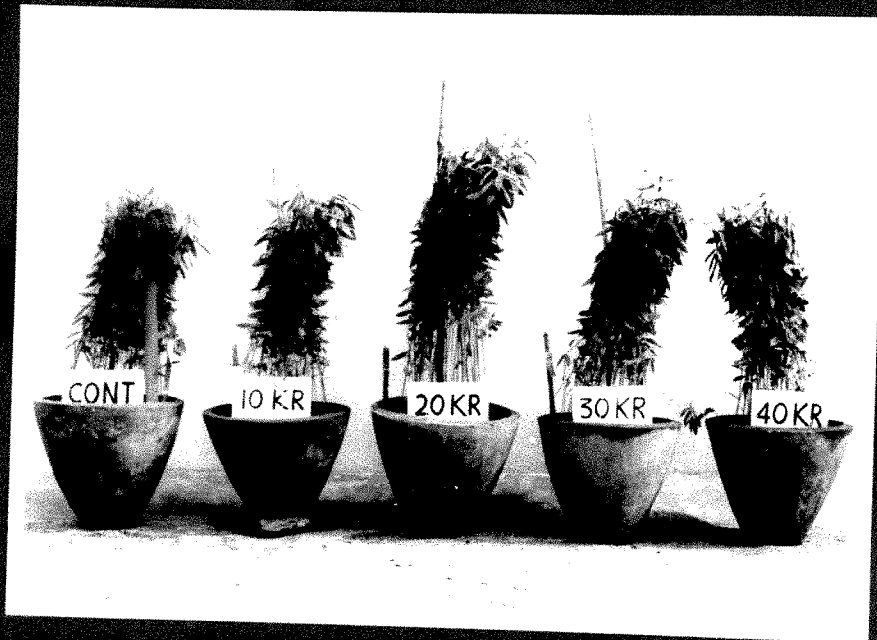


Fig. 23.

were pale green in colour with patches of normal colour here and there. The sectorial discolouration decreased after 30 days of growth in 20, 30 and 40 KR doses. The initiation of verigated branches has been observed from the axi~~l~~<sup>l</sup> of the leaves which were shown chimeric nature.

(c) Growth parameter :

The growth in cm. as recorded every week, after the seedling emergence of  $\sqrt{\text{V}}$ -irradiated plants of C. juncea are represented in Table 9 and Fig.22.

The general, pattern of growth did not changed ~~much more in C. juncea after  $\sqrt{\text{V}}$ -rays treatment (Fig.22).~~ Exceptionally, in 20 KR dose the plants have attained highest growth (Figs. 22, 23). Percentage of cotyledonary ~~emergence~~<sup>c</sup> was much more in 10 and 30 KR plants as compared to control and plants obtained after 20 KR and 40 KR dose. Initiation of flowering has been recorded simultaneously in control, 20 KR and 40 KR plants, where as the plants obtained after 10 KR and 30 KR treatments have shown delayed tendency of initiation of flowering (Table 10).

(d) Leaf area :

The average leaf area, average number of leaves per plant and the total leaf area per plant of

Table 9 : EFFECT OF  $\gamma$ -IRRADIATION ON GROWTH (HEIGHT)  
OF Crotalaria juncea ( $M_1$  generation).

Treatment	<u>Height (cm) at week intervals.</u>						
	1	2	3	4	5	6	7
Control	7.5	21.56	33.5	43.8	54.5	64.5	75.0
10 KR	7.5	20.9	30.0	39.0	50.5	61.5	68.0
20 KR	8.6	28.7	40.5	54.5	69.0	81.5	87.5
30 KR	7.9	23.9	34.5	43.0	55.0	68.0	78.0
40 KR	7.9	23.5	34.6	42.0	54.5	64.5	71.0

Table 10 : EFFECT OF  $\sqrt{\text{V}}$ -IRRADIATION ON DIFFERENT MORPHOLOGICAL PARAMETERS OF Crotalaria juncea.  
(M<sub>1</sub> generation).

Parameters	Treatment			
	Control	10 KR	20 KR	40 KR
1. Leaf area : i) Average leaf area cm <sup>2</sup>	19.88	21.05	27.1	21.57
ii) Number of leaves per plant.	36.	36.	40.	39
iii) Total leaf area per plant cm <sup>2</sup> .	715.7	757.8	1084.	841.2
2. Stomatal frequency cm <sup>2</sup> .				
i) Upper epidermis	143	131	107	167
ii) Lower epidermis	250	202	190	262
3. Initiation of flower after sowing (number of days)	33	40	33	33
4. Percentage of pollen fertility.	97	96	90	78.5

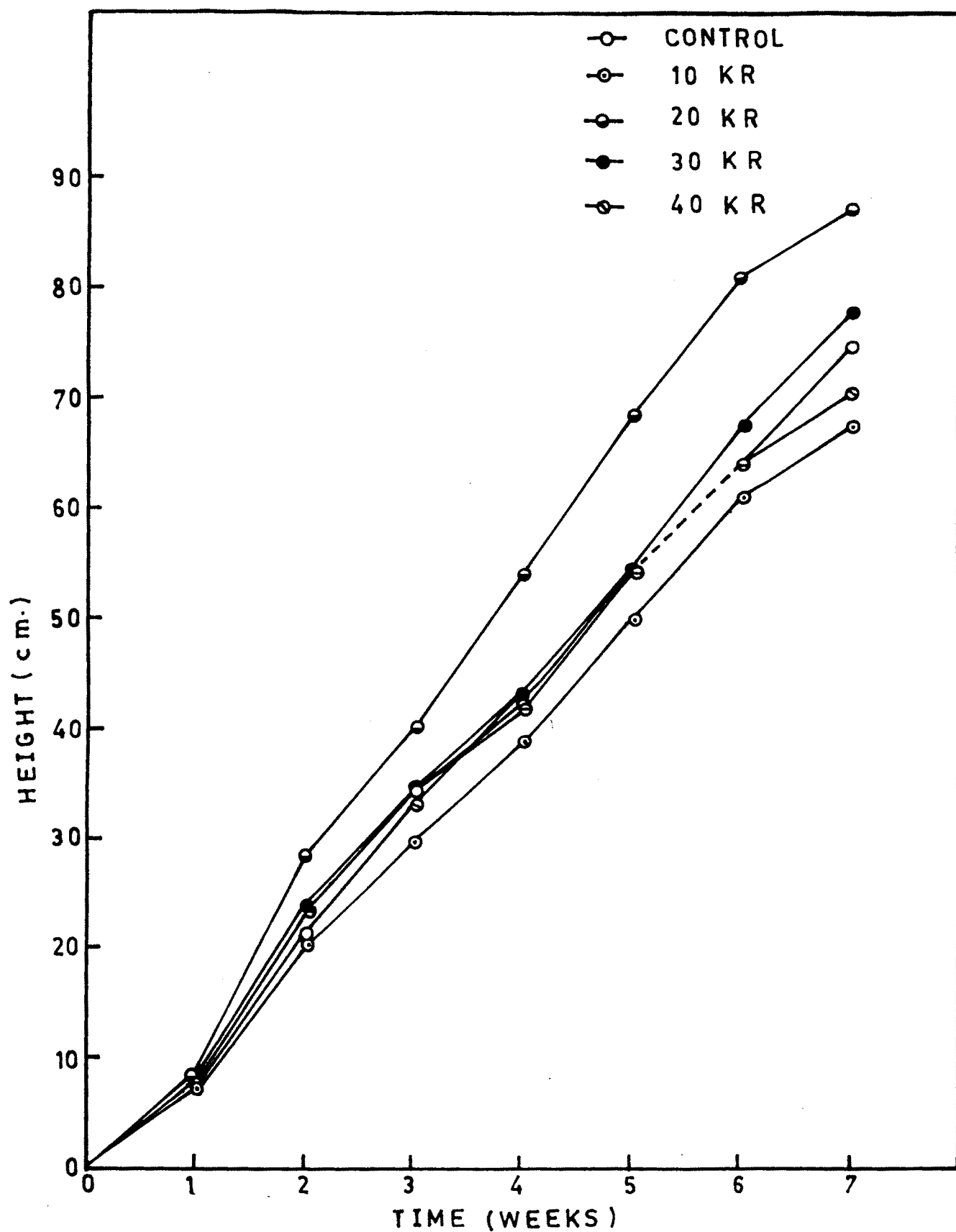


FIG. 22 : EFFECT OF  $\gamma$ -IRRADIATION ON GROWTH RATE OF Crotalaria Juncea L. (M<sub>1</sub> GENERATION) .

✓-irradiated is given in the Table 10.

From the table it is clear that, the decreasing order of the average leaf area ( $\text{cm}^2$ ) is as follows, 27.1, 21.57, 21.05, 20.73 and 19.88 in 20 KR, 40 KR, 10 KR, 30 KR and control plants respectively. The number of leaves per plant is as 43, 40, 39, 36 and 36 in 30 KR, 20 KR, 40 KR, 10 KR and control plants respectively. The total photosynthetic leaf area ( $\text{cm}^2$ ) per plant is in decreasing order as 1004.0, 391.4, 841.2, 757.8 and 715.7 in 20 KR, 40 KR, 10KR and control plants respectively.

(e) Stomatal frequency :

The stomatal density of leaves treated with increasing doses of ✓-irradiation in this species of Crotalaria is given in the Table 10.

It is clear from the table that the stomatal density went on decreasing as the irradiation dose increased up to 30 KR. At the highest dose chosen i.e. at 40 KR the stomatal density increased considerably, i.e. 167 per  $\text{cm}^2$  on upper epidermis and 262 per  $\text{cm}^2$  on lower epidermis as against 143 per  $\text{cm}^2$  on upper and 250 per  $\text{cm}^2$  on lower epidermis in control plants.



(f) Pollen fertility :

In  $\gamma$ -irradiation treatments of C. juncea the pollen fertility has been recorded in the Table 10.

It is clear from the table that the pollen fertility linearly decreases with increasing dose of  $\gamma$ -radiation. The percentage of pollen fertility being 97, 96, 90, 87.2 and 78.5 in control, 10 KR, 20 KR, 30 KR and 40 KR plants respectively.

(2) Physiological parameters :

(a) Moisture percentage

The effect of  $\gamma$ -irradiation on moisture percentage in C. juncea is depicted in Table 11.

The table indicates that there is no significant effect of  $\gamma$ -irradiation as far as the moisture percentage is concerned. In root and stem the moisture percentage is more than that of control plants in all the doses, where as in case of leaf it is just the reverse i.e. in control plants the moisture percentage is highest. If we consider the moisture percentage of entire plant, the sequence of decreasing order is as, follows 10 KR, 40 KR, 30 KR and 20 KR plants.

The decrease or increase in moisture percentage is very marginal one. The highest being 80.54% in 10 KR

Table 11 : EFFECT OF  $\sqrt{\text{V}}$ -IRRADIATION ON DIFFERENT PHYSIOLOGICAL PARAMETERS OF Crotalaria juncea  
(M<sub>1</sub> generation)

Parameters	Treatment			
	Control	10 KR	20 KR	40 KR
1. Moisture.				
a) Moisture percentage of				
i) Root	74.06	74.32	73.13	75.5
ii) Stem	79.8	81.8	79.9	81.2
iii) Leaf	85.8	85.5	84.5	85.0
b) Moisture percentage of the entire plant.	79.88	80.54	79.17	80.43
2. Chlorophyll.				
a) Chlorophyll "a"	161.0	156.4	161.24	159.5
b) Chlorophyll "b"	73.8	73.4	78.29	70.316
c) Chlorophyll "a"/Chlorophyll "b"	2.04	1.99	2.0	2.2
d) Total chlorophylls.	259.8	234.8	239.5	229.8
3. Nitrogen.				
a) Nitrogen content of				
i) Root	0.525	0.312	0.194	0.437
ii) Stem	0.925	0.575	0.675	0.70
iii) Leaf	2.07	4.18	3.05	3.18
b) Nitrogen content of the entire plant.	1.17	1.69	1.3	1.44

\* Values expressed as mg <sup>100</sup>-<sup>1</sup> g fresh tissue.

+ Values expressed as g <sup>100</sup>-<sup>1</sup> dry weight.

and lowest percentage being 79.17 % in 20 KR treated plants.

(b) Total chlorophylls :

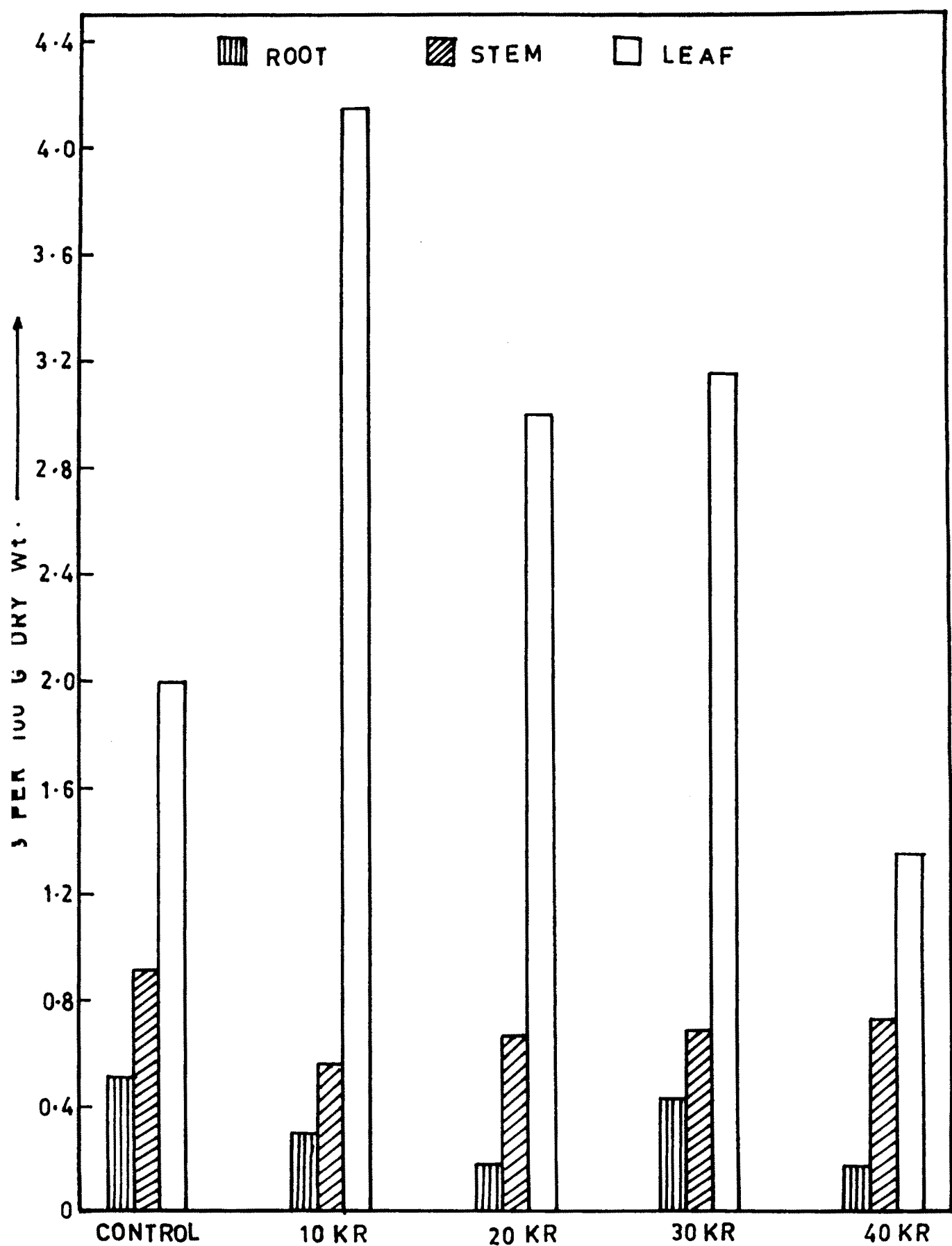
The effect of the increasing doses of  $\gamma$ -irradiation on the total chlorophylls of C. juncea is given in Table 11.

The table reflects that there is no any correlation between the radiation doses and the total chlorophylls. It is also clear from the table that in all the  $\gamma$ -irradiated plants the total chlorophylls is less than that of the control plants. The lowest amount (211.4 mg/100 g fresh tissue) of chlorophyll is observed in 40 KR treated plants which is the highest dose of  $\gamma$ -irradiation in the present investigation as against the high amount (239.83 mg/100 g fresh tissue) in control plants.

(c) Nitrogen :

The nitrogen level in C. juncea treated with increasing dose of  $\gamma$ -radiation is given in the Table 11 and Fig. 24.

The table and figure indicates that the  $\gamma$ -irradiation treatment has effected in lowering the nitrogen content in root and stem compared to the



**FIG. 24 : EFFECT OF  $\gamma$ -RADIATION ON NITROGEN CONTENT OF *Crotalaria Juncea* L. (M<sub>1</sub> GENERATION)**

control plants where as in leaf there is an increase in nitrogen level (4.18 g/100 g dry tissue) at the lower dose of irradiation (10 Kr); and decrease in the level (1.35 g/100 g dry tissue) at the highest dose chosen (40 KR). If we consider the total nitrogen of the entire plant the decreasing sequence is as follows 1.689, 1.439, 1.306, 1.173 and 0.755 g/100 g dry tissue in 10 KR, 30 KR, 20 KR, control and 40 KR treated plants respectively.

How this  
decreases  
plants

#### D. Discussion.

The results of germination study and survival percentage carried out in irradiated seeds of Crotalaria juncea are given in Table 8 and Fig. 16. It is clear from the table that the germination in the irradiated seeds started on the same day as that of control and also the rate of germination, survival percentage is not much affected by the treatment. It is also noticed that the process of germination and growth of radicle is triggered in all irradiated seeds compared to control (Fig. 17). Thus to get LD 50 in this species much higher doses than that of 40 KR is to be tried. However higher doses are known to cause larger damages than lower ones, and there are always chances of getting more beneficial mutations at lower doses. A number of studies have been conducted on radio sensitivity of various crop

plants (Sparrow and Woodwell, 1962; Sparrow, 1965; Sparrow and Sparrow, 1965; Singh, 1970) and on the inhibition of plant growth (Gray and Scholes, 1951; Ehrenberg and Dietrich, 1955; Ehrenberg, 1955; Davies, 1961 and Davies, 1968) by  $^{60}\text{Co}$  V-rays. Survival percentage reported in the present investigation indicates that the radio sensitivity of this species is much higher. Randelia (1980) found that in C. striata irradiation from 10 KR to 90 KR had no adverse effect on germination though the seeds of 100 KR took more time to germinate. This is in agreement with the present investigation that the genus Crotalaria is more radio resistant.

In the present investigation only few doses (10, 20, 30 and 40 KR) have been tried to induce mutations in C. juncea. Zhatov (1979) reported effect of lower doses of  $\gamma$ -rays on hempseeds and obtained stimulation of germination and seedling vigour. Bairathi and Nathawat (1979) reported ineffectiveness of lower doses of  $\gamma$ -rays to induce chromosomal aberrations in root meristems of C. juncea and they reported that 80-100 KR doses are toxic to this plant system. The results obtained in the present investigation are in conformity with the above findings.

It is evident from the literature reviewed by Gustafson (1947), Fujii and Matsumura (1958) and

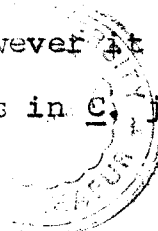
Matsumura and Fujii (1959) in most of the species studied, germination percentage decreased with increase in dose of radiation. Singh and Godward (1974) reported 100 % germination at all doses ranging from 2 to 500 K rad in Elusine coracena. However Shamsi et al., (1978) reported stimulation and early germination in Vicia faba following  $\gamma$ -irradiation of seeds. Guimaraes (1978), while studying the effect of  $\gamma$ -irradiation on rice seeds reported that survival was not affected by increased  $\gamma$ -ray doses. Constantin et al. (1976) reported that survival was unaffected by  $\gamma$ -irradiation doses less than 70 KR in Glycine max. Swiecicki (1981) observed in pea that increasing doses of irradiation caused delay in germination. From the present investigation it seems that germination and survival percentage is depended on doses of  $\gamma$ -rays and doses vary from species to species and cultivar to cultivar.

Number of damaging effects caused by  $\gamma$ -irradiation on germination have been recorded by various workers. It is known to cause disruption and disorganisation of tunica layer and there by inhibit germination (Chauhan and Singh, 1975). High dose of irradiation has been shown to be impairing mitosis or sometimes virtual elimination of cell division in meristematic zone of germinating seeds (Cherry and Hageman, 1961). Woodstock

and Justice (1957) have shown that irradiation causes respiratory inhibition too. On the other hand there are reports suggesting that irradiation causes dormancy break and stimulation in germination. (Sparrow and Singleton, 1953; Sparrow, 1954; Skok et al., 1965; Torne, 1965; Torne and Raut Desai, 1975). An inverse relationship between seed water content and sensitivity to sparsely ionizing radiations was first discovered by Caldecott (1954) and Ehrenberg and Nybom (1954). Later Caldecott (1957) has shown that seeds of 4% water content exhibited more damage if they were soaked in O<sub>2</sub> bubbled water after irradiation than if they were soaked in N<sub>2</sub> bubbled water. The influence of water content on seed radiosensitivity was extensively studied during early 1960's (Conger, 1961; Osborne et al., 1963 and Milan et al., 1965). Seed moisture percentage of C. juncea was found to be 6.483 which is beyond the limit reported by Coldecott (Loc. cit.) and still showing higher percentage of germination after higher dose irradiation. Thus Crotalaria is a genus which is more radio resistant than the others.

Lower doses induce early DNA synthesis and stimulated germination of seeds by acting at the physiological level, while higher doses cause damage to DNA (Dnyansagar and Tarar, 1971). However it is interesting to study the same hypothesis in C. juncea

why





which germinates after higher doses of irradiation.

Cotyledons were changed into variously deformed shapes with higher doses and even at 10 KR. Their length and breadth also varied, more or less similar observations were made by Lefort and Ehrenberg (1955), Mericle and Mericle (1957), Moutschen (1958) and Saric (1958) in barley. In these cases clefts were found to have occurred in the coleoptile when the seeds of barley were irradiated. In experiment with wheat sprouts, it was established by Vasilev (1961) that if 1 was assumed to be radiosensitivity of coleoptile the 6 was that of leaves, 16 of the primary roots and 18 of the side roots. In the present study sectorial discoloration was observed in C. juncea from 20 KR onwards and it was persisted upto 30 days of growth. Sybenga (1964) observed sectorial discoloration of leaves in irradiated Crotalaria intermedia. Bairathi and Nathawat (1974) studied morphology and anatomy of polycotylous and twin seedlings of C. juncea from natural population. They reported hemitricotylous, tricotylous and tetracotylous and twin seedlings, most of them show normal growth and reproduction. But in few connations of growing points, stem fasciation and whorled phyllotaxy were observed. In the present study tricotyledonous nature as well as seed with two radicles (Fig. 18) is observed after  $\sqrt{\text{ }}$ -ray treatment, may be due to change in growing points.

Similar type of results have been obtained by Singh (1974) in jute after  $\gamma$ -irradiation treatment.

It is evident from the Table 9 and Figs. 22 and 23 that even with increasing doses of  $\gamma$ -irradiation the general pattern of growth didnot change much, except for 20 KR dose. Similarly the height attained by control and 40 KR plants is almost same. However increased growth is observed in plants obtained after 20 KR treatment. Similar results were obtained by Tarav and Dnyansagar (1979) while studying effect of  $\gamma$ -rays and EMS on growth and branching in Terneria ulmifolia. They further reported the stimulation in seedling growth in case of lower expositors of  $\gamma$ -rays and height of the plant was more than that of the control. Zhatov (1979) observed similar pattern in hemp seeds treated with  $\gamma$ -rays. Premsekhar and Appadurai (1981) while studying the effect of  $\gamma$ -rays on Cajanus cajan observed that lower doses of  $\gamma$ -rays (10-15 KR) are effective to stimulate the growth. Khan (1981) studied the effect of  $\gamma$ -rays on seed germination, seedling growth in mung bean and observed that growth of seedling decreased with increase in doses of  $\gamma$ -rays. Bhattacharya (1977) <sup>Ref.</sup> showed that low dose (10 KR) improved the growth, yield and compositional characters of plants Glycine max grown from irradiated seeds and observed that higher doses showed progressive inhibition. Subramanian (1979) has

obtained similar results in species of Vigna after  $\gamma$ -irradiation.

Rabindra (1966) obtained two varieties of Corchorus olitorius by irradiation which on an average flowered earlier than the control and had greater plant to plant variation. Ramakanth et al., (1977) induced polygenic variability in field bean (Dolichos lablab) with  $\gamma$ -rays and observed early flowering in significantly many treatments. Rao and Rao (1978) reported early flowering in Abelmoschus esculentus induced by 8, 9 and 10 KR treatments, while flowering was delayed in 5 and 6 KR treated samples. Stepanenko and Regir (1982) reported early flowering in Calendula officinalis after  $\gamma$ -rays treatment. Similarly in the present investigation early flowering has been recorded in 20 KR and 40 KR plants with that of control. However in 10 and 30 KR the vegetative growth is extended upto 43rd days and then flower initiation, reproductive phase started. It is evident from the present investigation and foregoing discussion that induction of early flowering is characteristic mutation induced by physical mutagens in different plant systems. However such type of mutations are of prime importance in legumes which are used as food, feed and fodder. The variants obtained after 10 and 30 KR treatment are of use as a fibre and green manure plant where vegetative phase has prime importance. However the

results obtained in the present investigation are of  $M_1$  generation and they need further confirmation in successive generations.

From Table 10 one can conclude that irradiation effect is stimulatory at lower doses as far as leaf area is concerned. In 20KR the total photosynthetic leaf area has increased considerably over control. The increase in leaf area naturally counts for the productivity of the plant.

It is interesting to note that, though the leaf area is increased in 10, 20, 30 and 40 KR plants. The increase in stomatal frequency per unit area is observed only in 40 KR plants. The number of stomata decreased considerably in 20 and 30 KR plants over control. Rao and Rao (1978) observed no significant variation in stomatal index after X-ray treatment in ~~Abelmoschus~~ Abelmoschus esculentus. However in the present investigation though the plants obtained after 40 KR treatment are showing higher frequency of stomata per unit area should be studied in further generations to confirm the trait.

Pollen fertility decreased with increase in dose, but the decrease in 10, 20 and 30 KR plants is not significant, while 40 KR showing pronounced effect. It has been reported by Randelia (1980) in C. striata and C. retusa after  $\gamma$ -ray treatment that sterility was greater

than the frequency of chromosomal aberrations. Thus along with the abnormally dividing cells forming sterile pollen it can be assumed that even genic mutations and physiological changes must have played an important role in pollen sterility as suggested by Das (1957) in barley exposed to X-rays. The results of present investigation are in confirmity with those of Katiyar and Roy (1974, 1975), Katiyar (1978), Khan (1981) and Appadurai (1981), that  $\gamma$ -rays induced pollen sterility and is dose dependent and always higher than the detectable meiotic abnormalities. Earlier belief, that induced pollen sterility is mainly due to a large number of meiotic abnormalities during microsporogenesis, requires modification. Gaul (1964) suggested that interchanges played an important role in the induction of sterility and the sterility might be treated as more reliable index of chromosomal mutation than the detection of inter change multiples during meiosis.

It seems from the present investigation (Table 11) that the moisture percentage at  $M_1$  generation level in C. juncea has not been affected drastically. In general higher moisture percentage is recorded in 10, 30 and 40 KR plants, but increase is marginal, where as decrease in 20 KR plants is also near. Moisture characteristic varies from plant to plant and from place to place. However in the present investigation the general trend

of moisture percentage in  $M_1$  and  $M_2$  of DLS treated plants and  $M_1$  generation of irradiated plants is almost similar, indicating that the conditions provided for plant growth are more or less same. Moisture is a characteristic which deflects according to the agronomic conditions as well as chemical constitutions of the plants. The general trend of increase in moisture percentage is useful to screen the plants for the same in  $M_2$  generation. Green manure produced by this biomass will have maximum water content which helps to restore the fertility of the soil and to maintain the flora and fauna of the problematic soil. It is interesting to note here that the trials conducted at saline fields have also shown promising results in improving the texture and the general characteristics of the soil. It is also observed that the plants are more tolerant to saline conditions and also to drought. The plants obtained after 30 KR treatment thrived well under such conditions. Thus it indicates that  $\gamma$ -irradiation will be of use to improve C. juncea with different dimensions.

Total chlorophyll content after  $\gamma$ -irradiation is not increased or decreased significantly in C. juncea ( $M_1$  generation). There are reports by various workers that chlorophyll is a characteristic feature which responds immediately to mutagenic treatment. It is believed that the alteration in chlorophyll synthesis of

leaves obtained after irradiation may be due to auxin synthesis (Kuzin, 1956; Hagen and Gunckel 1958). Giacomelli et al., (1967) reported the primary effect of radiation was the development of meristematic cells and the effect on auxin supply may be a consequence of the same. Singh (1971) has observed in maize that above a threshold value (20 KR) chlorophyll content decreased gradually, but even at 40 KR the total chlorophyll content remained significantly more than the control. Rao and Rao (1978) observed in Abelmoschus esculentus increases in the chlorophyll content of leaves in plants of 4 KR and 7 KR treatment, while a dose of 8 KR resulted in more than 50% reduction of content over the control. Similar results have been obtained by Gupta and Yashvir (1974-1975) and Yashvir (1975) in A. esculentus. Foregoing discussion suggests that total chlorophyll content is dependent on the dose and on the radiosensitivity of that genome. In the present investigation the dose administered to Crotalaria juncea is below the LD 50 (Table 8 and Fig.16) and mutations obtained are less in number as chlorophyll content is considered.

Nitrogen level of the plant is one important factor which provides a clue to the overall metabolic status of the plant C. juncea is utilised as a green manure which contributes maximum nitrogen to the soil under reclamation. With the above view in mind the whole

plant analysis is carried out to determine the level of nitrogen. The nitrogen level in the plants obtained after irradiation treatment has shown increase upto 30 KR treatment, where as sudden drop is observed in the plants obtained after 40 KR treatment. This indicates that radiation is useful to produce a strain having higher nitrogen level.

Singh (1970) while studying the effects of irradiation on protein and aminoacid content of maize leaf had found that protein content increased with the dose of irradiation and heighest content was obtained in 30 KR irradiation leaves at ear formation stage. It was also observed that protein content of maize leaves increased with advance in age irrespective of treatment. Dahiya (1974) reported improvement of mung bean through induced mutations by  $\gamma$ -rays. The treatment with 70 KR was found to be more effective than 30 KR. Similarly high protein mutants of winter fodder barley has been obtained by Yankulov et al., (1982) after seed treatment with  $\gamma$ -rays. They observed the mutant with 1-4% higher protein content and besides high protein content (17.37%), lysine was 5.96 gram per 100 grams of proteins. G Giacomelli et al., (1967) reported depression of protein synthesis caused by irradiation. Similarly Schaefferbeke-Sacre (1977) has observed change in nitrogen content in  $\gamma$ -irradiated Jerusalem artichoke. Thus from the foregoing discussion it is clear that irradiation helps to increase



the nitrogen content of some genome and vice-versa.  
Our observations are in agreement with those  
suggesting increase in nitrogen content after  
✓-irradiation. It will be worth to screen the mutants  
having higher nitrogen, protein content in successive  
generations.