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V Yadav
Department of Botany,
Hislop College, Civil Lines,
Nagpur, Maharashtra, India

Correspondence
V Yadav
Department of Botany,
Hislop College, Civil Lines,
Nagpur, Maharashtra, India

Effect of gamma radiation on various growth parameters and biomass of *Canscora decurrens* Dalz

V Yadav

Abstract
In the present study, seeds of *C. decurrens* were exposed to gamma radiation at doses (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50kR) to examine its effects on growth traits and morphological variation. The various physiological effects were observed on germination, no. of plant survived, shoot induction, ht. of plant, ht. of root, no. of nodes and leaves, fresh weight and dry weight. Gradual reduction was observed in no. of plant survived with increased the doses similarly earlier germination and shoot induction were recorded at lower doses. Lower doses 10, 15, 20 and 25kR showed stimulation in seedling shoot and root length, no. of nodes and leaves as well as fresh weight and dry weight where as reduction was observed in 30, 35, 40, 45 and 50kR. Various morphological variants like stem thickening, more no. of nodes with shorter internodes, increase in leaf size and area were also observed etc. This paper summarizes and discusses the effects on different growth parameters and biomass attainment through the use of ionizing radiation in *C. decurrens*.

Keywords: Mutation, gamma radiation, doses, *Gentianaceae*, *C. decurrens*

1. Introduction

Canscora decurrens (2n =38) (Syn. of *C. diffusa*) belongs to family *Gentianaceae*, which includes 99 genera and approximately 1,736 species [1, 35]. This genus is included in the famous "*shankhpushpi*" group of plants including *Convolvulus microphyllus*, *Evolvulus alsinoides* and *Clitorea ternatea*, which are used as ingredient in formulation used to improve intelligence, memory, and other higher mental function [18]. The entire plant is used for the treatment of nervous disorders like epilepsy, insanity, nervous debility etc. The alcoholic extract of *C. decurrens* is able to cure a number of disorders of CNS particularly concentration problem, learning disorders, weak school performance, refusal of school, dyslexia, mental retardation development retardation and delayed speech development, aberration, mental dementia and age related dementia, Alzheimer's diseases, dysmnesia, fragile x syndrome, depression, schizophrenia, affective psychosis, mania, states of anxiety and panic, hyperkinetic behavioral disorder among children and adults, hallucination, compulsive washing behavior abnormalities, anorexia nervosa, lack of motivation, borderline syndrome autism, bipolar affective disorder, mental impairment, stage fright, nightmare, tic disorder and lack of self confidence. There is a great need for therapeutic enrichment, particularly for the treatment of disorders in the intellectual area, to produce drugs and /or substances, which not only exhibit good efficacy, but also have a wide range of indications as well as few or no side effects [4].

However in practice, main constraint in production of *C. decurrens* plants on mass scale (for mutation breeding experiments as well as commercial purpose) is inability to cultivate these plants in pots or field due to very high specificity of growth conditions. Further being an annual plant biomass can be obtained only once in year. These facts reduce the economic value of *C. decurrens* for future commercial exploitation. Hence the main objective of the present study was to grow these plants in controlled conditions so as to obtain supply of material throughout the year. Further attempts were needed to undertake plant improvement through mutagenesis (gamma radiation) in order to increase the biomass for its commercial exploitation [38].

Mutation is a sudden heritable change in an organisms and generally a structural change in gene. Mutation change in the base sequences of genes are known as gene or point mutation. Some mutation produced by change in chromosomes, structure, or even in chromosome number is known as chromosomal mutation Mutagens are known to affect the morphology, physiology and genetic behavior of plants. Mutations either spontaneous or caused by mutagens are stable and heritable changes in the genetic material and its behavior in plants [20]. Induced mutagenesis has been widely used for improvement of potential traits of various economically important plants. The prime strategy in mutation breeding is to upgrade well adapted plant varieties for commercial value addition [23].

Radiation treatment has been the most frequently used method to develop direct mutant varieties, accounting for about 90% of obtained varieties with gamma rays and 22% with x rays [12]. Gamma radiation (GR) is the most widely used physical mutagen in crop improvement. Gamma rays are electromagnetic radiation (ionizing radiation) with the highest energy level. Gamma rays affect the DNA molecule by rupturing of hydrogen bonds between the base pairs, breaks in one or both of the DNA strands. This impact of radiation on DNA produces multitudes of effect at morphological, growth, physiological, cytological and gene expression levels in treated plants. These effects may be deleterious (lethal) or significantly positive which is a matter of chance. The plants showing desired effects can be selected for further breeding [8]. Gamma rays belongs to ionizing radiation and interact with atoms or molecules can damage or produce free radicals in cells. These radicals can damage or modify important component of plants eg. dilation of thalakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds [14]. The economical and more effective features of gamma rays due to their high penetration power helps in their wider application for the improvement of various plant species compared to other ionizing radiations. Gamma irradiation has a profound influence on plant growth and development by inducing genetical, cytological, biochemical, physiological and morphogenetic changes in cells and tissues depending on the levels of irradiation [2].

2. Materials and method

Preliminary survey in local areas indicated that *C. decurrens* is available in wild condition around Nagpur. Mature seeds of *C. decurrens* were collected in the month of March from Devlapar area near Nagpur, Maharashtra. The seeds were kept in zip lock pouches at room temperature for further use. Dry seeds were exposed to radiation at 10, 15, 20, 25, 30, 35, 40, 45, and 50kR doses (Source: ^{60}Co, P.G department of chemistry, RTM Nagpur university). The treated seeds (7mg) were soaked in minimum quantity of water for 6hrs, then surface sterilized in laminar air flow with 1 wash of mercuric chloride (0.01%) and thrice with distilled water and seeds were inoculated on MS basal media and kept under controlled condition for 3 month for further germination and growth. Effect of gamma was studied on various growth parameters like germination, shoot induction, no. of plant survive, ht. of shoot length, root length, no. of node, no. of leaves, fresh weight and dry weight and total chlorophyll. Chlorophyll content was estimated by spectrophotometer and absorbance was recording at 663 and 645nm wavelength. Quantity of chlorophyll was calculated by following formula [27]:

mg chlorophyll a\g tissue=12.7(A_{663})-2.69(A_{645}) ×v\1000×w

mg chlorophyll b\g tissue=22.9 (A_{645})-4.68(A_{663}) ×v\1000×w

Total chlorophyll\g=20.2(A_{645}) +8.02(A_{663}) ×v\1000×w.

In gamma radiation morphological abnormalities like, increase in intermodal distance, leaf area, size, colour, texture, margin, size of stomata, branching, thickening of stem, thickening of root, multiple shoot induction were recorded The data was recorded at the end of 3 month in all cases.

2.2 Statistical Analysis

The mean and ANOVA was calculated by using SPSS version 21 with one-way analysis followed by Tukeỳs multiple comparison test and significance was determined at $p<0.05$.

3. Results

3.1 Effect of gamma radiation on germination, shoot induction and survivility.

In lower doses (10, 15, 20 and 25 kR) germination was enhanced by 2-3 days. In 20 and 25kR it was earliest i.e initiated on 5th days and continued till 7th day of inoculation. Germination was almost complete with in 6 to 7th days in these doses. In 10 and 15 kR on the other hand germination was initiated on 6th days and continued for 8-9 days which is comparable to control. However germination rate was significantly lowered as the process continued till 13th days (in 30, 35 and 40 kR). In the highest dose (50kR) germination was extended and initiated and complete during 12th and 13 days.

In control around 54 days were required for apical shoot induction, while in 10 and 15 kR it was enhanced to 50 days. 20 and 25 kR can be claimed as stimulatory because shoot formation was observed in 45 days ie, 9 days earlier than control. As the doses increased further the apical growth became slow as indicated by the fact that 60 and more days required for 1st pair of leaf to appear. The highest dose (50kR) the apical shoot induction was prolonged indefinitely. These result clearly indicated that 20 and 25 kR gamma doses had stimulatory impact on early growth while higher doses (30 to 50kR) proved to be inhibitory or lethal.

The sensitivity of dry seeds of *C. decurrens* to gamma radiation was evaluated by comparing the mean number of plants survived per 7mg weight of seeds.

Data in Table.1 indicated that in all doses (10 to 50kR) there is definite decrease in plant survival as compared to untreated seeds. Control exhibited survival of 133 plants and it was reduced to 28 in highest gamma rays dose (50kR).The reduction in survival is dose dependent i.e with increasing dose there is gradual decrease in survivality. Near 50% plants survived in 30 and 35kR indicating LD_{50} range of gamma rays in *C. decurrens*. Higher doses proved to be lethal with only 43.3, 35 and 28.6 no. of plants in 40, 45 and 50 kR respectively. As all the plants were grown in controlled condition, variability due to environment condition is least and the decreasing trend in survival can directly be considered is gamma radiation effect.

Table 1: Effect of Gamma radiation on plant survival in *C. decurrens*.

Doses	Mean no. of Plant survived
(kR)	
10	128
15	123.33
20	103
25	97.33
30	70
35	61.67
40	43.33
45	35
50	28.67
Control	133.33

3.2 Effect of gamma radiation on different growth parameters (fig.1 and 2)

If the total vegetative growth of plant is divided in to 4 variable namely shoot length, root length, no. of nodes and no. of leaves, all variables show significant variation in different doses of gamma rays (Table.2).

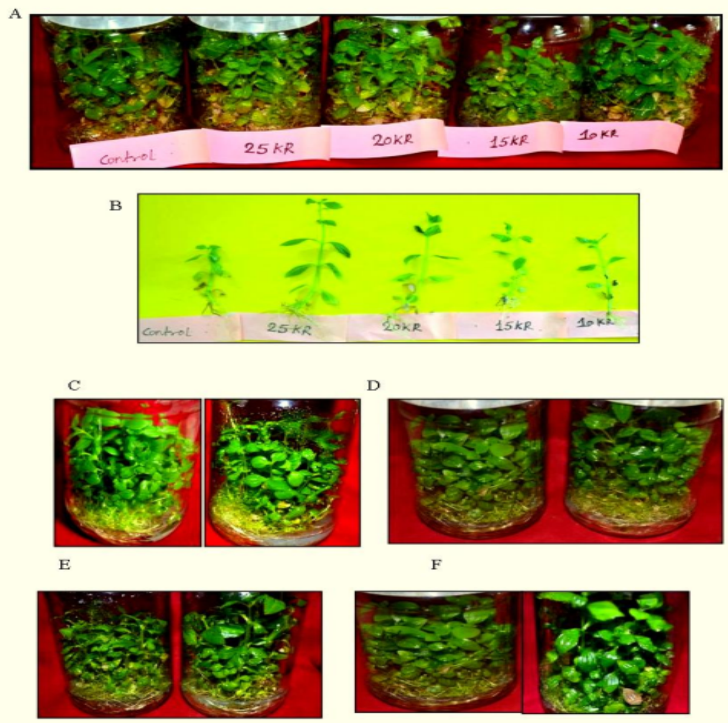


Fig 1: Effect of different doses of gamma radiation on the vegetative growth of *C.decurrens*. A- Comparative plant growth B: Comparative shoot length, C-control+ 10kR, D-control+ 15kR, E-control+ 20kR, F-control+ 25kR

Table 2: Effect of gamma radiation on different growth parameters and biomass of *C. decurrens*.

	Parameters \Doses	Shoot length (cm)	Root length (cm)	No. of nodes	No. of leaves	Fresh weight (g)	Dry weight (g)
Positive growth	10(kR)	4.60±0.02^{e}	4.17±0.12^{f}	4.50±0.07^{d}	10.99±0.15^{d}	11.16±0.13^{c}	0.74±0.04^{d}
	15	5.28±0.03^{f}	4.54±0.04^{f}	5.00±0.04^{e}	11.97±0.10^{e}	9.72±0.94^{b}	0.56±0.03^{bcd}
	20	6.09±0.03^{g}	5.45±0.05^{g}	5.97±0.04^{f}	14.05±0.10^{f}	13.53±0.55^{d}	0.73±0.06^{cd}
	25	8.11±0.04^{h}	6.20±0.05^{h}	7.69±0.14^{g}	16.83±0.32^{g}	9.45±0.69^{b}	0.52±0.05b^{cd}
Negative growth	**Control**	**3.56±0.03^{d}**	**3.33±0.08^{e}**	**3.16±0.04^{b}**	**8.33±0.10^{b}**	**11.20±0.23^{c}**	**0.51±0.01^{bc}**
	30	2.00±0.05^{b}	1.69±0.06^{b}	2.95±0.02^{b}	7.94±0.05^{b}	0.78±0.05^{a}	0.21±0.02^{a}
	35	2.03±0.05^{b}	2.31±0.11^{d}	3.20±0.07^{b}	8.43±0.17^{b}	1.30±0.23^{a}	0.45±0.09^{b}
	40	2.47±0.05^{c}	1.97±0.11^{bc}	3.05±0.03^{b}	8.18±0.19^{b}	0.43±0.03^{a}	0.14±0.02^{a}
	45	2.26±0.06^{c}	2.19±0.09^{d}	3.62±0.10^{c}	9.27±0.19^{c}	0.38±0.03^{a}	0.12±0.02^{a}
	50	1.24±0.07^{a}	1.16±0.11^{a}	2.38±0.11^{a}	6.041±0.26^{a}	0.29±0.02^{a}	0.05±0.01^{a}

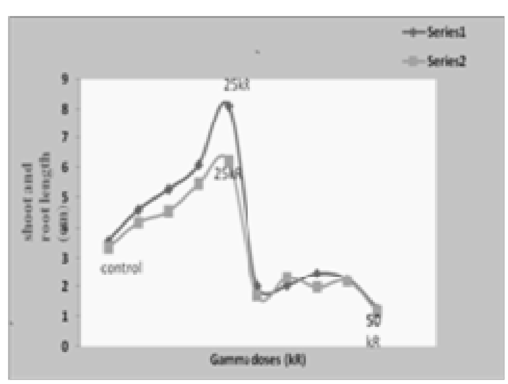
The data shown are means±SD of three replicates. Mean with in column followed by same letter are not significantly different at p≤ 0.05. Different letter a, b, c, d, e, f, g, h, bcd, cd and bc denote significant difference (p≤ 0.05) between different treatment

The results signify mean values of all variables, the data of which was recorded at the end of 3 months. In control the shoot length, root length, no. of nodes and no. of leaves were found to be 3.5cm, 3.3cm and 3.16 and 8.33 respectively.
Two distinct trends towards positive and negative growth with respect to control are clearly observed, in *C. decurrens* after gamma irradiation.

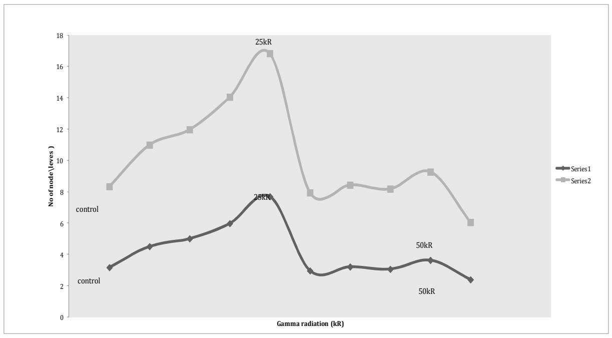
1. In lower doses (10, 15, 20 and 25kR) the values of shoot and root length, no. of nodes and leaves are significantly higher than control. Hence these doses will be called as stimulatory (positive growth).
2. In higher doses (30, 35, 40, 45 and 50 kR), significant reduction in plant growth was observed. These doses are grouped and called as inhibitory (negative growth).

Within lower doses groups as the dose increases there is gradual increase in the growth. Maximum growth was found in 25kR with 8.11cm (shoot length), 6.20cm (root length) 7.69(no. of nodes) and 16.83(no. of leaves). It should be noted that these is more than 2 fold increase in the growth in 25 kR as compared to control.

Sudden drop in all growth parameters with small increase in dose from 25 to 30kR is the most highlighted feature of radiation treatment. There is prominent decrease in shoot length (from 8.11to 2.0cm); root length (6.2 to1.6cm), no. of nodes (7.69to 2.95) and no. of leaves (16.8to 7.9) (Graph 1 and 2).



Graph 1: Effect of gamma radiation on the shoot and root length [series1-shootlength, series2-root length]



Graph 2: Effect of gamma radiation on no. of node and leaves [series1-no. of node, series2-no. of leaves]

If all higher doses are compared it is found that there are no significant variation in all growth parameters in 30, 35, 40 and 45kR with average values ranging from 2.0 to 2.47 cm (shootlength); 1.69cm to 2.3cm (rootlength), 2.9 to 3.6 (no. of nodes and 7.9 to 9.2 (no. of leaves).Extreme reduction in growth was recorded in highest gamma dose (50kR) (fig.2).

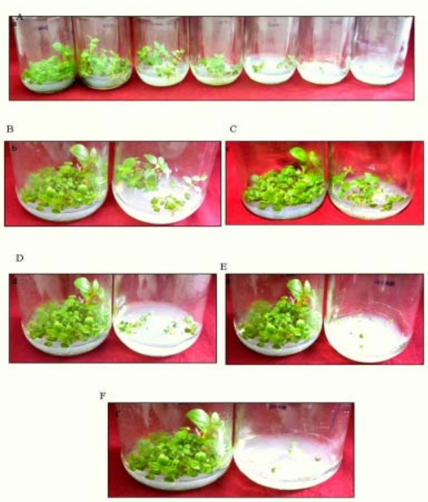


Fig 2: Effect of higher doses of gamma radiation on the vegetative growth of *C.decurrens*. A- Comparative plant growth in 25,30,35,40,45 and 50 kR;B-control+30kr;C-Control+35;D-control+40;E-control+45kR ;F-control+50kR ;

Fresh weight measurement also showed 2 distinct trends. Severe reduction in FW could be seen in higher doses (30kRto 50kR) as compared to control. Among higher doses the variations obtained in FW are not statistically significant. In lower doses the maximum FW was obtained in 20kR (13.53gm) as compared to control (11.20 gm). 15 and 25 kR had similar effect of FW while lowest dose (10kR) was in effective as the FW value (11.16) is similar to that of control (11.2 gm)

The maximum fresh weight was observed in 20kR(13.53gm) where as lowest exhibited in 50kR(0.29gm) as compared to control (11.20gm).Dry weight of whole plants of *C. decurrens* after gamma irradiated was significantly decrease from control (0.51gm) to 50kR (0.05gm) except at 35 kR with 0.45gm DW. Extra ordinary increase was observed in 10kR (0.74) and 20kR (0.73gm).While 15kR and 25kR treatment showed values (.56gm and 0.52gm).Similar to control.

3.3 Leaf morphology

Different doses of gamma rays produced variable effect in the leaf morphology which did not show dose dependent effect. Rather there was promising increase in size of leaf at 20 and 25kR. There is tendency towards normal size in lower (10 and 15kR) as well as marginally higher doses (30 to 40kR). Very high doses showed great reduction in leaf size.

Leaf colour in all gamma-treated plants exhibited broad spectrum shades of green colour irrespective of doses. In 45kR, 30kR dark green leaves were observed while in 10,40 50kR it was similar to that of control (green).The remaining doses produced very light green coloured leaves. Leaf shape showed variation in the form of obvate and reniform leaves as against normal ovate shape with either crenate (normal) or serrate (abnormal) leaf margin.

Stomata are typically anomocytic where as at higher doses anisocytic or paracytic types were also obtained. Sometimes variation in stomatal distribution was observed in the form of paired or clumps of more than 2 stomata (fig. E,F) (Table.3).

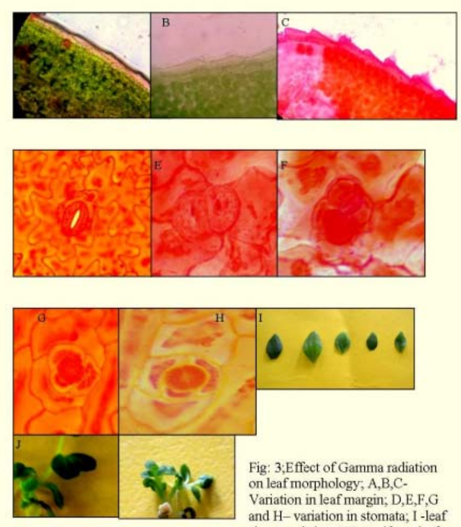


Fig: 3;Effect of Gamma radiation on leaf morphology; A,B,C-Variation in leaf margin; D,E,F,G and H– variation in stomata; I -leaf shape and size; J,K-remiform leaf, and small leaf.

Table 3: Effect of gamma radiation on leaf parameters of *C. decurrens*

Doses ↓ \Parameters →	Size of leaf	Colour of leaf	Shape of leaf	leaf margin	Type of stomata.
10 kR	Small	Green	Ovate	Crenate	Anomocytic
15	Small	LightGreen	Ovate	Crenate	Anomocytic
20	Large	Light green	Obovate	Crenate	Anomocytic
25	Large	Light green	Obovate	Crenate	Anomocytic
30	Small	Dark green	Obovate	Crenate	Anomocytic
35	Small	Light green	Obovate	Crenate	Anisocytic
40	Small	Green	Obovate	Crenate	Anomocytic
45	Very small	Dark green	Reniform	Serrate	Anisocytic
50	Very small	Green	Reniform	Serrate	Paracytic and hexacytic
Control	Small	Green	Ovate	Crenate	Anomocytic

Large- above 1cm, small- below 0.7cm,very small- less than 0.2cm

3.4 Morphological abnormalities (fig.4)

Certain morphological variants after gamma treatments were observed. Some plants showed extra growth activity in the form of stem thickening, more no. of nodes with shorter, internodes, pronounced auxiliary branching, thick and large leaves (fig A, 25kR).

In contrast, in the same treatment a lanky mutant with slender stem, thin delicate leaves and lateral branching was observed (fig. B).Highly deformed seedling with thick, fleshy stem, dark green undeveloped leaves and thick and green roots were obtained showing very high degree of genotoxicity of higher dose (fig.G). Reni form leaves, excessive elongation of internode, excessive root growth early flowering with large, 4 petal flowers are some other deformities induced by various doses of gamma rays (figA-M).

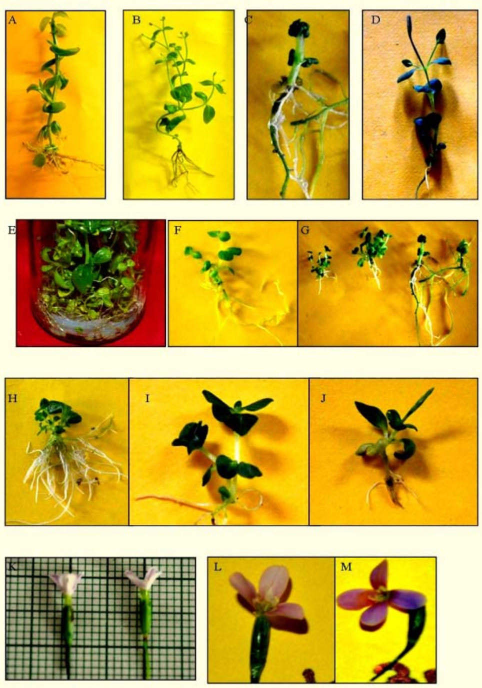


Fig: 4: Morphological abnormalities in gamma radiation; A,B– increasein no. of node, and branching;C– thickening of shoot; D– change in phyllotaxy,E- increase in intermodal distance;F-remiform leaf;G– absence of shootapical, and reduced leaf,; H– increase in no. of root,I-shape of leaf ,J– early bud formation;KLM– four petal, bigger in size. flower .

3.5 Chlorophyll content:

Total chlorophyll content showed great variation in the gamma treated plants. Invariably it was less in all doses as compared to control value (3.07mg).Among different concentration, higher doses (35,40,45 and 50kR)induced higher chlorophyll (19.64,19.72,27.6 and 27.28mg respectively) content than lower doses (10,15,20,25 and 30kR)(Table.4).

Table 4: Effect of Gamma radiation on chlorophyll content.

Doses (kR)	Chlorophyll a (mg)	Chlorophyll b (mg)	Total chlorophyll (mg)
10	2.81	2.036	4.80
15	7.51	5.3	12.84
20	7.31	5.202	12.4
25	10.76	6.36	17.1
30	0.25	14.36	14.08
35	14.21	5.19	19.64
40	14.5	5.14	19.72
45	2.97	24.82	27.6
50	2.72	24.32	27.28
Control	3.85	27.24	31.07

In control chlorophyll-b is significantly more (27.24mg) than chla (3.85 mg). However this trend was opposite in 10, 15, 20, 25, 35 and 40kR where chla is more than chlb. Further increase or decrease in chl-a and chl-b content does not correlate with concentration of gamma radiation.

4. Discussion

It is known that various rays and chemical matters have positive or negative effects on living organisms. This effect can occur both spontaneously on nature and artificially by mutagens. In this study, we have tested the effect of gamma radiation on the different growth parameters like germination, shoot induction, ht. of shoot and root, no. of leaves, no. of nodes, fresh and dry weight.

Theoretically exposure to gamma rays produce negative effects on plant growth and development. The extent of growth reduction varies with plants species and dosimetry. Hence radio sensitivity of tissue (plant) must be determined by exposing the seeds to different intensities of radiation and by studying ‘dose- response’ effect on several growth traits. Scientific literature on radiation mutagenesis abound with such radiation induced growth inhibition [3, 6].

Contradictory to this, the present investigation revealed 2 contrasting patterns namely radio stimulation (increase in all growth attributes) and radio inhibition (reduction in growth parameters or inducing lethality. From the data obtained (Table.2) it is very much clear that gamma rays had highly significant impact on all growth parameters in *C. decurrens*. Increase in shoot length, root length, no. of nodes and leaves was observed in low doses than control plants. Similar correlation i.e “low dose- high growth” was also observed by [22] in *Molluccella lavis*; [10] in *cucurma longa*; [31] in *Hibiscus sabdariffa* and [28] in *Dracaena serculosa*. In the present study 10, 15, 20kR doses showed significant stimulation in shoot and root length and leaf number. Hence these doses can be grouped as “sublethal” doses. At higher doses (30, 35, 40, 45 and 50 kR) there is severe reduction in plant height and all other growth parameters indicating lethal effect by these doses. Seedling root and shoot length was declined with increasing dose in all varieties of Basmati. Here radiation showed highly significant negative correlation with seedling shoot length (-0.998) and seedling root length (-0.941) exhibiting dose dependent response. Root length was affected more than shoot length. Higher doses of gamma rays produce a broad range of negative activities at physiological levels like, water exchange, leaf gas exchange, enzymatic and hormonal imbalance, altered protein synthesis etc [37].

Low dose of gamma rays was most positively effective on subsequent growth of plant [30, 32]. There are 3000 reports on stimulation by low dose irradiation and termed this effect as “radiation hormesis.” Hormosis in general is defined as excitation or stimulation by small doses by any agent in any system. Here “low dose” is a relative term which is defined as threshold dose that marks the boundary between bio positive and bio negative effects. Most physiological reactions in living cells (plant, bacteria, invertebrate and vertebrates) are stimulated by ionizing radiation. This radiogenic metabolism includes enzyme induction, photosynthesis, respiration, cell division and growth [17].

In the present study high and low doses of radiation elicited opposite reactions. Stimulation response increased gradually with increasing dose showing highest stimulation at 25kR. After that with every increase of gamma dose by 5kR, growth inhibition was prominently observed. It is interesting to note that the values for all growth attributes are nearer to control values. It can be recalled that 50% survival was observed in

35kR (Table.1) It clearly indicated 35kR to be threshold levels in *C. decurrens* under given experimental conditions.

The possible reasons for the observed results in *C. decurrens* are explained below-

1) Radiation is a potential hazard because it can damage DNA and impair physiological processes leading to cytotoxic effects. Large no. of free radicals or reactive oxygen species (ROS) is produced in excess in the irradiated tissue. They include H-hydrogen free radical, HO-hydroxyl free radical, H^+- hydrogen ion, H_2O_2- hydrogen peroxide. The ROS react with almost all, structure and functional molecules in cells like protein, lipid, nucleic acids etc OH cause peroxidation of membrane fatty acids forming peroxyl (ROO^-) and alkoxyl (RO-) radicals resulting in loss of cellular compartmentation and its attack on protein greatly alters properties and functions. Damage caused to DNA induce chromosomal and gene mutations [36, 38]. Plant in turn takes up counter measure to protect themselves from this damage [16, 15] for the purpose plants bring about changes by altering the hormonal signaling network in growing tissue or by increasing antioxidative capacity of cells. Increase in antioxidative capacity can be done in 2 ways-

1. Enzymatic- Enzymatic defense is based on the increase in the activities of well known enzymes like catalase, peroxidase (Glutithion peroxidase, GH or ascorbate peroxidase ASH), superoxide dismutase (SOD). These enzymes replenish the pool of scavengers and help in transportation and elimination of free radicals [7].
2. Non enzymatic- In this response vit-E, carotenoids, flavonoids. Flavonone etc. have a special role as scavenger [21].

Phenolic compounds provide strong tool by which the plants can remove these free radicals. Efficient scavenging of ROS by phenolic compounds has been found to reduce uv-radiation stress especially “Flavonoid- peroxidase” reaction acts as mechanism for H_2O_2 scavenging and thus flavonoid acts as detoxifying agent.

In vitro studies have confirmed that flavonoids and related compounds can directly scavenge O_2 (superoxide), H_2O_2 and HO. radicals produced after tissue is exposed to stress conditions [29]. Phytochemical analysis in *C. decurrens* indicated that whole plants of *C. decurrens* is rich source of phenolic components including flavonoids, flavonone, xanthone more than 16 xanthone derivatives have been reported in *Canscora* species [5]. It might be possible that the physiological damage produced by gamma doses treatment in *C. decurrens* might have eliminated by the phenolic compounds and “low dose” stress might have acted as growth elicitor.

Attention should also be paid to the unusual phenomena of “post germination apical growth inhibition in *C. decurrens*. Ideally shoot growth takes place immediately after cot leaf development if light condition are normal [25, 33]. In *C. decurrens* prolongation of apical growth in situ, ex situ (laboratory and field) and in vitro condition is a common features even in normal plants(prolongation period may rich 90 days and more).In gamma rays treated plants this period is a crucial period during which the repair and recovery machinery operates in full swing. The irradiated *C. decurrens* plants might have overcome this stress period positively making the low doses not only non-lethal but acted as hormetic(stimulatory).To conclude the concept of hormesis provides a major rule in biology stating “small doses are stimulatory large doses depress” and replaces the fear “all radiation is harmful”.

4.1 Seedling and leaf abnormalities

Size of leaf majorly contributes to the vegetative biomass of plants. Gamma rays showed induced variation in the leaf size in *C. decurrens*, with smallest (2-3mm) to largest (more than 1cm) leaf sizes, light green leaves, variable shapes and leaf margin. In *Cucurma longa* gamma irradiation had significant effect on average length and width of leaves, inducing larger sized leaves at lower doses while higher doses had inhibitory effect on length of leaves [10].Gamma rays also influence density, distribution and type of stomata [11, 24].

There are many reports in the literature emphasizing shoot \ plantlet formation from different explants but only few studies showed direct shoot regeneration from seed (embryo) under *in vitro* conditions [6]. The mature embryos have high capacity for shoot formation and generally single shoot embryo is formed in MS basal media without any phytohormones [19]. Lagre no. of extreme abnormalities in leaf and seedling suggested strong interaction and impact of mutagen on these genes. Although these mutants are not desirable in the context of present study they provide a measure of sensitivity of plants to mutagen.

4.2 Chlorophyll content

The fundamental significance of photosynthesis in any commercial plants is its direct impact on biomass production (Fw\ Dw) and economical viability. High rates of photosynthesis maintain the plants against stressful eventualities. A considerable amount of research has been focused on evaluation of effect of different mutagens on chlorophyll content of treated plants. Stimulatory effect of low doses of gamma on chlorophyll amount has been reported by [14].Higher doses of gamma radiation and other mutagens destroy the pigments namely Chla, Chlb and Cartenoids [34].

However radio sensitivity of these pigment vary with experimental conditions which strongly suggested that chl-a and chl-b synthesis are independent of each other.

5. Conclusion

Gamma radiation effect showed two patterns (1) Increased in all growth parameters with increase in dose from 10 to25 kR. (2) Significant reduction of growth and higher lethality from 30 – 50kR. 25kR gamma irradiation was found to be most effective growth stimulating dose.

6. References

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