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Genetic studies on Jatropha Plant (Jatropha Curcas Lam) using diferent Gamma Radiation doses

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Abstract: The study aimed to compare, the effects of different doses of gamma radiation on germination survival percentage and growth of Jatropha plant, the seeds were treated with three doses of gamma radiation (5, 10 and 15 kr) and arranged in a randomized complete block design with three replications. The results showed significant differences among the three doses for seed germination after 7, 14 and 21 days from sowing in vitroand invivo experiments and on the other handplant height andno. of Leaves /plantwere recorded after 10,20, 30,100,120 and 130 days from sowing invivo experiments andthe low dose 5 kr seemed to have a stimulating effect on these traits, on the other hand the high dose 15 kr caused significant reduction as compared to the control for all studied traits.

Key words: Jatropha callus, gamma radiation, seed germination

i. INTRODUCTION

Jatropha curcas L. belongs to family Euphorbiaceae, native to South America attained significant importance for its seed oil, which can be converted to biodiesel, a renewable alternative energysource. Easy adaptation to different kinds of marginal lands, drought endurance, and its short interval time to first yield make this species more attractive for cultivation as a biodiesel plant and its protein composition [13]. Curcas is one of the most valuable crude drugs of primitive times and is still widely used in modern medicine. It has natural distribution covering the new tropic form. and now distributed throughout the entire tropics of Africa and Asia as well [7]. In recent years, this plant has received extensive attention of many scientists in view of its great economic importance, medicinal significance and for its seed oil as commercial source of fuel [3]. The superior quality oil can be extracted from the seeds, which can be used, as a mixed fuel for diesel/gasoline engines [21]. The genus Jatropha comprises around 160 to 175 species, typical of tropical and warm climates. The species can be monoecious or dioecious, trees, shrubs rhizomatous subshrubs, or geophytes, and herbs, including some annual taxa [4] and [9]. The plant can be used for basket making, as living fences and in folk medicine, as ornamental. Almost all the species are diploid, with 2n=2x=22 chromosomes.

ii. MATERIALS AND METHODS

The present investigation was carried out by cooperation between genetic unit Botany Department Faculty of Agriculture, Al-Azhar University Cairo, Egypt and Biotechnology Research Group Botany Department, National Research Centre (NRC), Dokki Egypt.

A-Material:

Healthy seeds of Jatropha species (*Jatropha curcas* L.) were purchased from Luxor city, Luxor governorate, Egypt that harvested at April 2013.

B –Methods:

1- Irradiation

Seeds of Jatropha were subjected to three doses of gamma rays (cobalt 60) (5, 10, and 15kr). Irradiation was carried out at National Center for Radiation Research and Technology, Cairo, Egypt.

2- Greenhouse experiment.

Irradiated seeds were sown directly in multi pot transplant trays, filled with a mixture of peat moss and sand (1:1) and arranged in a complete randomized block, the experimental design withthree replicationrandom samples from generation of M1.Plants were taken to study the effect of gamma irradiation on the following,germination percentage after 7, 14 and 21 days from culture and morphological characters as plant height (cm) and no. of leaves per plant after 10, 20, 30,100,120 and 130 days from culture comparing with control.

3- **Seed Surface sterilization**. Seeds coat were removed aseptically after washing one time using sterile – distilled water. Thereafter under aseptic conditions using Laminar air-flow cabinet, they were firstly immersed in 70% ethanol solution for 1 minute and then soaked by immersion in Clorox 30% (sodium hypochlorite v/v) at different time (table. 1), plus 2 drops of tween 20. Finally, it washed three times using sterile-distilled water.

C_2H_5OH 70 % Ethyl alcohol concentration (v/v)	Clorox
30% (15 minutes)	1 minute
30% (10 minutes)	1 minute
30% (20 minutes)	1 minute
30% (25 minutes)	1 minute
30% (30 minutes)	1 minute

4- In vitro Germination of Jatropha seeds.

Seeds were planted aseptically in culture bottles contained germination- medium solidified with 7gL-^{1} agar. The germination medium where sterilized by autoclaving at 121 ºC for 20 min. Seed cultures were maintained in dark at 27± 2 ºC for 11 days. Upon germination, seedlings were transferred to continuous light (2,000-Lux). After germination seed lings transferred to M.S [14]. Containing 30 gL-^{1} Sucrose and The PH was adjusted at 5.8; the medium was dispensed at 40 ml jars under cool white florescent light tubes. Morphological characters; germination percentage (in both *in vitro*and *in vivo*) at 7,14 and 21 days were taken. Plant height (cm) and number of leaves per plants were scoredat 10, 20, 30 100, 110,120 and130 days from culture*in vivo*.

5- Statistical analysis procedure

The experiments were subjected to completely randomized design. Each treatment was replicated three times. Values from triplicate determinations of each sample were averaged and represented as mean± standard deviation (s.d). The data were analyzed statistically by analysis of variance (AN.O.VA.), and the difference between the mean of samples were analyzed by least significant difference (LSD), test at a probability level of 5%, [18].

iii. RESULTS AND DISCUSSION

1- Seed Surface sterilization

- The effect of ethanol (C_2H_5OH) and on Clorox 30 % for seed surface sterilization.

Data in Table -1 shows the effect of ethanol (C_2H_5OH) (70 %) for1 minutes and also, illustrated onclorox 30 % at different time (10, 15, 20, 25 and 30 min)for seed surface sterilization. Also the relation between some disinfectant treatments, contamination free (%) and germination (%) of (*Jatropha curcas*) seeds.Data clearly showed that a suitable method to sterilizeseeds had been achieved, in which seeds sterilized with ethanol 70% for 1 minute followed by clorox 30% for 30 min.with two drops of tween-20 per 100 ml and contamination free % was 92.29%, whereas germination percentage was 97. 64 % after7 days from culturing.

Table (1): The percentage of seed sterilization of J. curcas with Ethyl alcohol (70%) for 1min. and Clorox (30%) at different time.

Ethyl alcohol C_2H_5OH70%	Clorox Concentration (v/v)	Contamination Free (%)	Germination (%) after 7 days
1 minute	30% (10minutes)	81.1	**48.9**
1 minute	30% (15minutes)	84.3	**52.5**

Ethyl alcohol C_2H_5OH70%	Clorox Concentration (v/v)	Contamination Free (%)	Germination (%) after 7 days
1 minute	30% (20 minutes)	86.14	**82.5**
1 minute	30%(25minutes)	88.59	**89.66**
1 minute	30% (30 minutes)	92.29	**97. 64**

2- Effect of gamma radiation on seeds germination.

Table-2 and Figure-1 showed that the germination percentage in the control (non-radiated seeds) as well as in thetreated seeds of *J curcas*on germination medium under *in vitro* condition. The differences in values of seedsgermination ratio due to gamma irradiation at different time intervals (7, 14 and 21th days after sowing) was highly significant at 5% level in seed germination under *invitro*condition. The data showedthat the highest germination ratio was (97.9%) obtained from radiated seeds with 5Kr, while the lowestmean germination ratio (77.08 %) with non-radiated seeds. Data appearing that radiation increased the germination ratio by 20.1%. [12]. reported that in okra (*Abelmoschus esculentus)* germination percentage generally decreased with increasing doses of gamma rays.while reduced germination percentage with increasing doses of gamma radiation has also been reported in Pinus. [19]. treated seeds of ***Jatropha curcas*** with gamma rays with 5, 10, 15In the laboratory germinationtest it was found that increase in concentration of gamma rays had anadverse effectSeeds treated with 25 Kr dose also showed a stimulatory effect and recorded57 % of seed germination as compared to other gamma rays treatments except for 5Kr dose [11]. reported that gamma irradiation interfered with the synthesis of enzymes and at the same timeaccelerated the degradation existing enzymes involved in the formation of auxinand thus reduces the germination of seeds. Reduced seed germination due to mutagenic treatments may be the result of damage of cell constituents at molecular level or altered enzyme activity

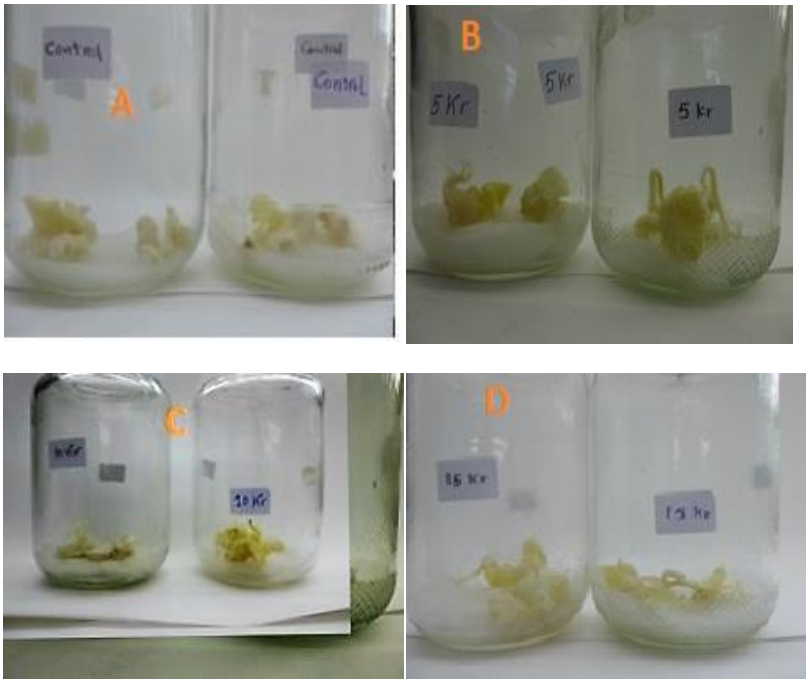

Figure (1) In vitro germination seeds showed (A- non-radiated seeds, B- radiated seeds(5kr), C- radiated seeds (10kr) and D- radiated seeds(15kr) of Jatropha on germination medium).

Table (2): In vitro germination seeds percentage of J. curcas treated with different doses of gamma radiation after 7.14 and 21 days.

Germinated seeds in vitro percentage of J. curcas				
Treatment	After7 day	After14 day	After 21 day	mean
Control	62.50%	81.25%	87.50%	77.08%
5kr	93.75%	100.00%	100.00%	97.9%
10kr	62.50%	87.50%	93.75%	81.25%
15kr	75.00%	81.25%	81.25%	79.16%
Kruskal Wallis Test	6.875	5.625	5.000	-
P VALUE	0.076 NS	0.131 NS	0.172 NS	-

Columns with similar letters are not significantly different according to LSD, NS= non –significant.

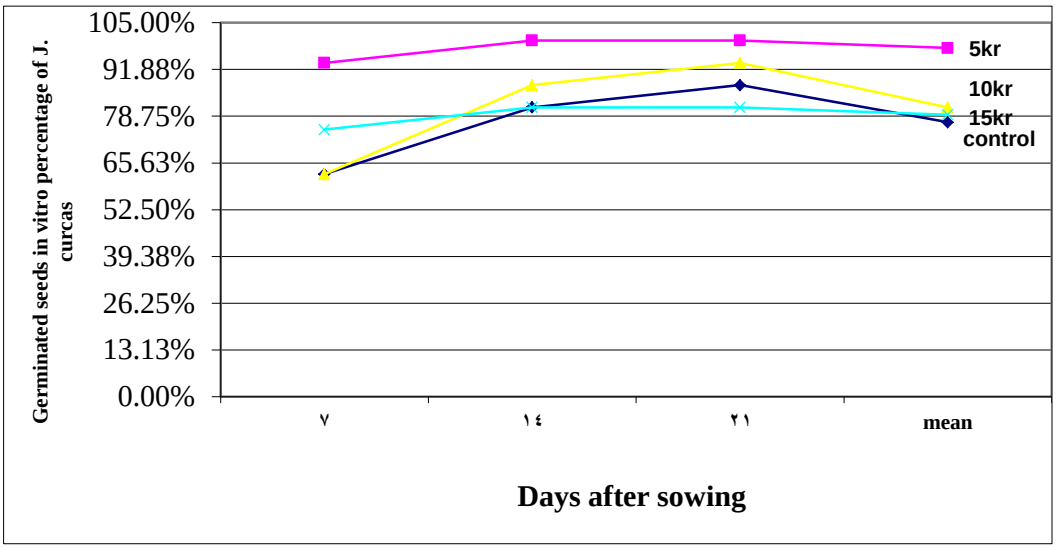

Fig. (1): In vitro germination seeds percentage of J. curcas treated with different doses of gamma radiation after 7.14 and 21 days.

Table-3 showed that the germination of radiated and non-radiated seeds on plastic trays filled with sand and beat-mos. mixture. The highest germination ratio (50%) was obtained from radiated seeds with 5Kr, while the lowest germination ratio (21.66%) was the control, (non-radiated seeds). Data appeared that radiation increased the germination ratio by 28.34%. There were highly significant differences in radiated J. curcas.

Table (3): Percentage of seed germination in vivoof Jatropha with different doses of gamma radiation.

Percentage of seed germination in vivo				
Treatment	After7 day	After14 day	After 21 day	Mean
Control	25.00%	20.00%	20.00%	21.66%
5kr	37.50%	56.25%	56.25%	50%
10kr	25.00%	31.25%	31.25%	29.16%
15kr	37.50%	43.75%	43.75%	41.66
Kruskal Wallis Test	1.475	8.204	8.204	-
P VALUE	0.688 NS	0.042*	0.042**	

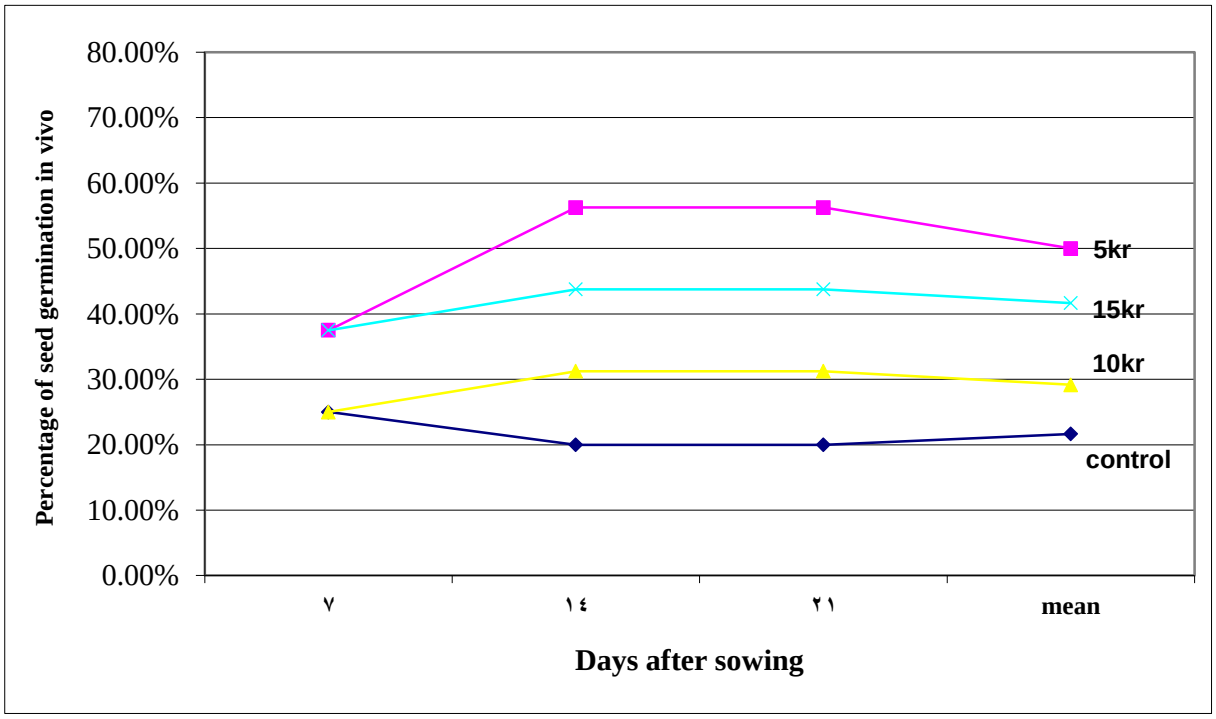

Fig. (2): Percentage of seed germination in vivoof Jatropha with different doses of gamma radiation.

Columns with similar letters are not significantly different according to LSD. NS= non -significant, * = significant at P < 0.05, ** = significant at P < 0. 01, *** = significant at P < 0.001. Results in table (3) demonstrated highly significant differences within treatments in vivo.

Table (4): Effect of gamma rays on plant height of J. curcas in vivo after (10, 15, 30, 100,120, and 130 days from sowing)

plant height (cm) in vivo						
	After 10 day	After 15 day	After 30 day	After 100 day	After 120 day	After 130 day
control	13.40±1.50 b	23.00±1.37 a	27.20±1.20 a	84.00±1.48 a	93.20±1.32 a	93.40±1.40 a
5kr	16.80±0.58 a	21.40±1.17 a	22.40±0.81 b	73.20±±1.32 b	90.80±0.80 b	92.60±1.22 a
10kr	12.20±0.74 b	13.60±0.68 b	17.60±0.75 c	62.20±1.43 c	77.20±1.39 c	77.00±0.95 b
15kr	12.00±0.63 b	8.80±0.73 c	14.60±1.50 c	44.00±1.38 d	72.00±1.22 c	61.80±1.62 c
F ratio	5.574	41.787	24.854	147.917	73.118	132.702
P VALUE	0.008*	0.000**	0.000**	0.000***	0.000**	0.000**

Columns with similar letters are not significantly different according to LSD. NS= non -significant, * = significant at P < 0.05, ** = significant at P < 0. 01, *** = significant at P < 0.001

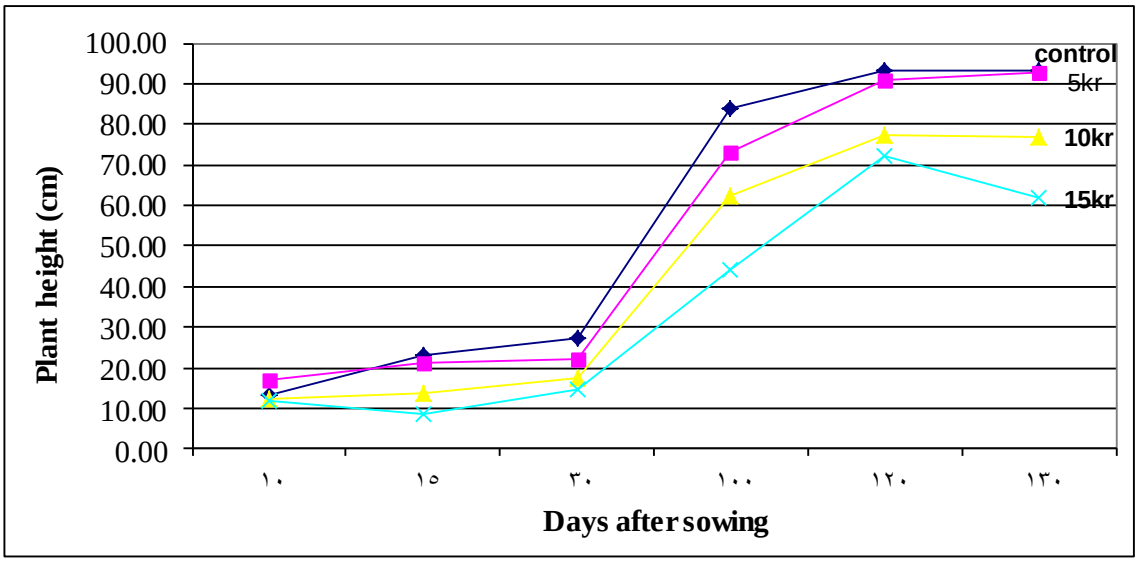

Fig. (3): Effect of gamma rays on plant height of J. curcas in vivo after (10, 15, 30, 100,120, and 130 days from sowing)

Table (5): Effect of gamma rays no. of leaves per plant in vivoat (10, 15, 30, and 100,120,130 days after sowing) on J. curcas.

no of leaves pear plant in vivo						
Treatment	after 10 day	after 15 day	After 30 day	After 100 day	After 120 day	After 130 day
control	3.00±0.45 a	3.80±0.20 a	6.40±1.20 a	25.40±1.48 a	37.20±0.66 a	37.40±1.40 a
5kr	3.00±0.32 a	3.60±0.24 a	6.60±0.81 a	26.80±1.32 a	33.20±1.20 b	37.00±1.54 a
10kr	2.20±0.20 ab	3.80±0.20 a	6.00±0.75 a	21.60±1.43 a	26.00±0.32 c	27.60±1.33 b
15kr	2.00±0.00 ab	3.20±0.49 a	6.40±1.50 a	23.60±1.38 a	27.20±1.39 c	26.80±0.73 b
F ratio	3.255	0.842	0.288	1.252	28.122	16.405
P VALUE	0.043*	0.491NS	0.833NS	0.324NS	0.000**	0.000*

Columns with similar letters are not significantly different according to LSD. NS= non -significant, = significant at P < 0.05, ** = significant at P < 0. 01, *** = significant at P < 0.001

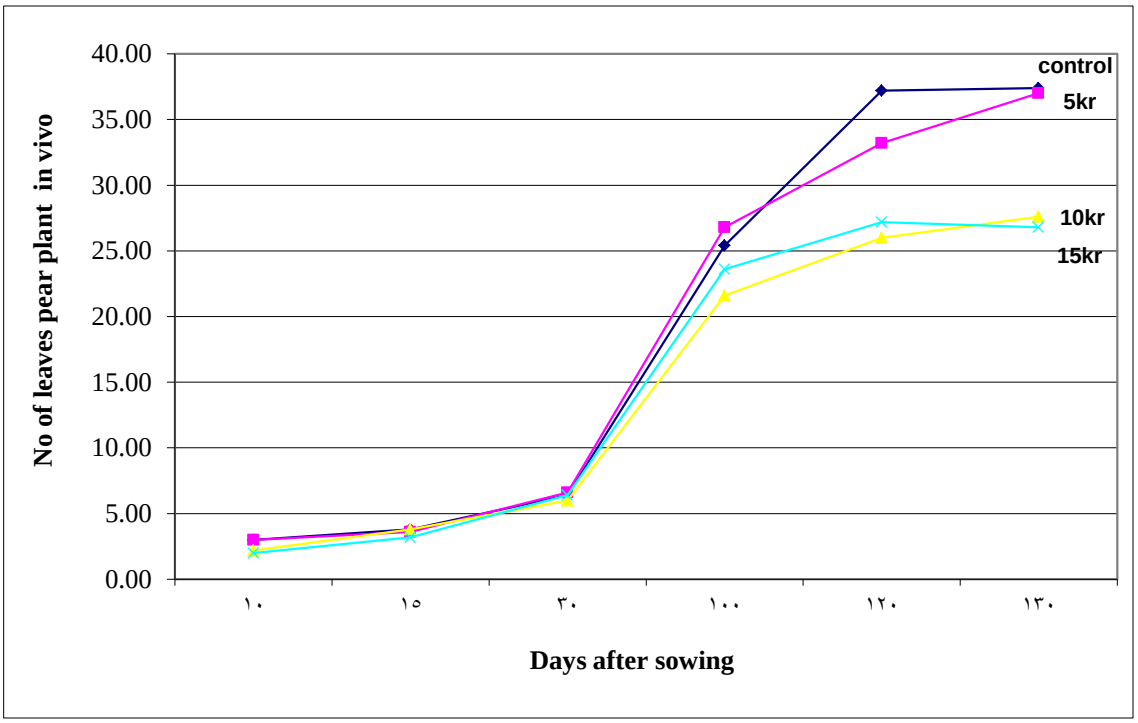

Fig. (4): Effect of gamma rays no. of leaves per plant in vivoat (10, 15, 30, and 100,120,130 days after sowing) on J. curcas.

It's clear from table (4) and (5) that gamma rays were drastically reduced the length of shoot seedling and vigor index and number of leaves pear plant inJ. curcas at higher doses / concentrations. This reduction increased by increasing reduction doses in sunflower [6]. The inhibitory effect of mutagens on the length of seedling was evident from the decrease in length of shoot by increasing the dose of gamma rays. The reduction of shoot length was attributed to the effects of mutagens on the physiological system [5]. Such a reduction in length of the shoot arising out of mutagenic treatments was previously reported in crop plants [16], [1] and [20]. The stimulatory effect was observed in lower doses of gamma rays on the length of shoot and seedling. The hypothetic origin of these stimulations by irradiation treatments was due to the increase in cell division rates as well as the activation of the growth hormone, e.g., auxin [22] and [8]. Table (4) showed that plant height at 130 days' maturity was 93.40±1.40 cm and was 92.60±1.22 cm in 8 5 Kr dose treatments, respectively while minimum plant height was observed in 15 Kr doses of gamma rays (61.80±1.62 cm). In the present study, gamma radiation at lower concentration has shown a stimulatory effect for plant height. [10], found that the radiation doses of 5, and 10 Kr had slightly reduced plant height while the other dose had no considerable effect on plant height. [2], and [15]. Reported that mutations were affected the plant height.

The result indicated that the mutagens could cause both positive and negative genetic variability in plant height (Table 5) also showed a maximum number of leaves after 130 days (37.40±1.40) on the control

followed by 5 Kr dose of gamma rays (37.00±1.54) and 27.60±1.33 at 10 kr and 26.80±0.73 at 15 kr doses. All the treatments of gamma rays revealed an inhibitory effect on the plant height as compared to control.

iv. CONCLUSIONS

From the previous results it could be concluded that lowest dose of gamma rays, (5 Kr dose) was found to be more effective on seed germination and showed a stimulatory effect as compared to the control while the highs dose (10 and 15 kr) treatments showed an inhibitory effect on all the parameters studied as compared to the control. The stimulatory effect at a lower dose is due to the fact that mutagens at lower concentrations stimulate the role of enzyme and growth hormones responsible for growth, while the inhibitory effect is due to the fact that biological damage this may be due to DNA damage such as breakage, inversion deletion of DNA structure, and even interaction with epigenetic processes could be useful for genetic improved plants for production of high seed yield.

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