Effect Of Ionizing Radiation On Some Characteristics Of Seeds Of Wheat

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ABSTRACT: Seed irradiation during pre-sowing processes is one of the most effective methods to improve the plant production. This investigation was carried out to determine the effects of ionizing radiation on germination and physiological characteristics of wheat seedlings. Therefore two separate experiments were, therefore, conducted 1. Using the filter paper method to assess the germination and rate and the initial seedling vigour indices I and II in the laboratory conditions. 2. Following a randomized complete block design with three replications in the field of the Nuclear Research Laboratory, IARI, New Delhi. Seeds of Wheat plant were irradiated with gamma rays at Control (0), 0.1, 0.15, 0.155, 0.16, 0.165, 0.17, 0.175, 0.18, 0.19, 0.20, 0.25 kGy on the Cobalt-60 Gamma irradiation chamber (GC-5000, dose rate 2.18 kGy/h) at the Laboratory. Irradiation doses depend on many conditions that involve exposure to the rays (type, quantity and radiation time) and behaviour of the irradiated environment (absorption capacity, physical, chemical and biological modifications, and secondary reactions) (Evangelista, 2000). The parameters studied were percent germination, plant biomass including root and shoot mass and plant height. To determine these effects, seeds were exposed to radiation with time period of 165, 247, 255, 264, 272, 280, 288, 296, 313, 330 and 412 seconds for the irradiated treatment as given above respectively. A dose-dependent increase in percentage emergence in radiated plants up to 0.17 kGy. At higher dose of 190 Gy, there was a drastic reduction in physiological characteristics of seedling of wheat. Shakoor et al., (1978) and Khalil et al., (1986) attributed decreased shoot lengths at higher doses of gamma rays to reduced mitotic activity in meristematic tissues and reduced moisture contents in seeds respectively. The study concludes that gamma radiation at a low dose stimulates, while a high dose (0.18 kGy and above) inhibits plant growth and development of wheat.

Index Terms: Ionizing radiation, wheat, percentage emergence, plant biomass, plant height.

1. INTRODUCTION

Ionizing radiation can safely and effectively eliminate the pathogenic bacteria from the food [11], disinfests the fruits and vegetables [11] [7], extend the shelf life of many products through ripening delay, inhibit the sprouting of bulbs and tubers [7] [5] and reduce or totally eliminate the parasitic microorganisms. Food conservation methods have been well accepted by the consumers. However, some disadvantages are frequently associated with them, especially regarding unwanted changes in the organoleptic characteristics and nutrient loss. It is well known that heat treatment can improve the nutritional value of foods. Similar effects of irradiation have been occasionally reported, but they are less common and usually less pronounced than the beneficial effects of heating [13]. Thermal treatment can cause significant deterioration in sensory properties of food. Mild treatments, such as pasteurization can cause substantial changes in the flavour of products such as milk. Slow freezing can alter texture of the vegetables, such as strawberries. Modern processing techniques such as the use of modified atmosphere cause minor changes in the sensory quality of the products but represent a higher cost and do not assure long shelf life. Food irradiation processes have been widely studied and are as well known as any other food processing method, such as dehydration and freezing [11].

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One of the main obstacles for the development of this technique in many countries is the mistaken ideas consumers have concerning excessive nutrient denaturation, along with the myth of food becoming radioactive and generation of toxic compounds. However, research results back to the 1950's have already shown the absence of radioactivity inducement in the food treated by ionizing radiations. The main advantages of irradiation are the small alterations in food components. Studies have shown that the macronutrients such as proteins, carbohydrates and fat are quite stable to the doses up to 10 kGy. Micronutrients, especially vitamins, can be susceptible to any food treatment method. The main purpose of cereal irradiation is decontamination and elimination of pathogenic microorganisms. The 10 kGy dose, accepted by Codex Alimentarius Committee (WHO, 1994) is very effective for the microbiological decontamination of wheat (*Triticum vilgare*), barley (*Hourdeum vulgare*) and sorghum (*Sorghum biocoler*), and does not provoke unfavourable nutritive effects to them. The small loss of nutrients (thiamine 22-33%, and riboflavin 10-16%) could be compensated by beneficial effects of processing [5]. Researchers with white rice fortified with folic acid, packed and later irradiated with necessary doses for insect disinfestations, showed vitamin retention in the food, with minimum nutrient losses. Losses in products based on crushed grains were bigger than in whole grains. However, this loss could be minimised by oxygen exclusion during the irradiation and storage. The effect of Co-60 gamma rays on the content of several B-vitamins in two varieties of Brazilian beans has been studied. Carioca (Phaseolus vulgaris L.var.Carioca) and Macacar beans (Vigna unguiculata L.Walp,var. Macacar) were irradiated at doses of 0, 0.5, 1.0, 2.5, 5.0 and 10 kGy, and subsequently stored at ambient temperature for six months. The content of vitamin B1, B2 and B6 was analysed by HPLC. Only slight changes were observed for thiamine and riboflavin, whereas a dose-dependent decrease was noted for pyridoxine, which, however, was significant only at the highest doses of 5 and 10 kGy. However, at the disinfestations dose up to 11 kGy, acceptable ratings were obtained for the sensory evaluation. In conclusion, for insect disinfestations of Brazilian beans, radiation processing could be a promising technology. Wheat is one of the first cereals known to have been domesticated, and wheat's ability to self-pollinate greatly facilitated the selection of many distinct domesticated varieties. Wheat (*Triticum aestivum*) improved by acclimatization, selection and hybridization has a very old historical background; to be unsatisfactory because of the limited genetic variation among the existing wheat population. Chaudhuri reported that the irradiation of wheat seeds during germination reduced its root and shoot lengths. Gamma rays belong to ionizing radiation and are most energetic form of such electromagnetic radiation having the energy level from around 10 KeV to several hundred KeV. Therefore, they are more penetrating than other types of radiation such as alpha or beta rays. Gamma radiation can be useful for the alteration of physiological characters. Gamma irradiation was reported to be a good procedure to improve the nutritional quality of broad bean. Significant positive effect of gamma irradiation at 0.15 kGy was reported on growth and development of okra.

2. MATERIALS AND METHODS

2.1 Seed irradiation treatment

Experimental Material
Seeds of HD 2967 of Wheat were procured from division of genetics and used as a plant material in this study.

Gamma radiation dose
Gamma irradiation was conducted using 60Co gamma source at a dose rate of 0.1, 0.15, 0.155, 0.16, 0.165, 0.17, 0.175, 0.18, 0.19, 0.20, 0.25 kGy.

2.2 Parameters

2.2.1 Growth attributes

2.2.1.1 Germination percentage
Seeds of all the radiated and non irradiated treatments were sown in sand in three replicates at 25 °C. Each replicate having 20-25 Seeds of all the treatment of gamma dose. The germinated seeds were evaluated initially at 5 days and finally at 10 days for normal, abnormal seedling, un-germinated and dead seeds (ISTA, 1985). Germination percentage was given on the basis of normal seedling only and percent germination was calculated by following formula:

$$\text{Germination \%} = \frac{\text{Number of seed germinated} \times 100}{\text{Total number of seeds}}$$

2.2.1.2 Seedling vigour
Ten normal seedlings from each replicates were taken at random and the shoot length and root length was recorded by linear scale. Seedlings were dried overnight in an oven set at 90 °C and the weight of 10 seedlings together per replicate was measured. Seedling vigour was calculated following Abdul Baki and Anderson (1973):

Vigour index I = Germination%×Seedling length (Root +Shoot)

Vigour index II = Germination% ×Seedling dry weight (Root +Shoot)

2.2.2 Physiological attributes

2.2.2.1 Biomass
Total biomass was determined at all the critical stages of plant growth. For this weight of leaf, stem and root were recorded and kept for drying in hot air oven at 70 °C for drying. After complete drying, dry weight was recorded. On the basis of observation the change in biomass was determined on per day basis.

2.2.2.2 Root mass (g)
Root mass per plant was measured by drying the roots at 60o C in a hot air over till constant weights were achieved. The root mass is expressed in g dw/plant.

2.2.2.3 Root length (cm)
Root length of plants was measured by using a ruler or scale and expressed as cm per plant.

2.3 Germination Test

Germination test were carried out at laboratory conditions. This experiment was conducted as wholly randomized design with 2 replications. Firstly, filters papers were soaked for 4-8 hours under tap water. Then seeds were placed on the top of the filter paper. Each filter paper with seeds were rolled and placed in a vessel containing distilled water. Readings were taken on 4^{th}, 6^{th}, 8^{th}, 10^{th} and 12^{th} Day respectively.

3. RESULTS

3.1 Effect of pre-sowing seed treatment of gamma irradiation on germination raised on germinating paper at 25 ± 2ºC and percentage emergence of wheat.

3.1.1 Seed germination
Gamma irradiation did not affect seed germination at the lower doses. At higher doses of 0.19 kGy and beyond, it was significantly affected over control.

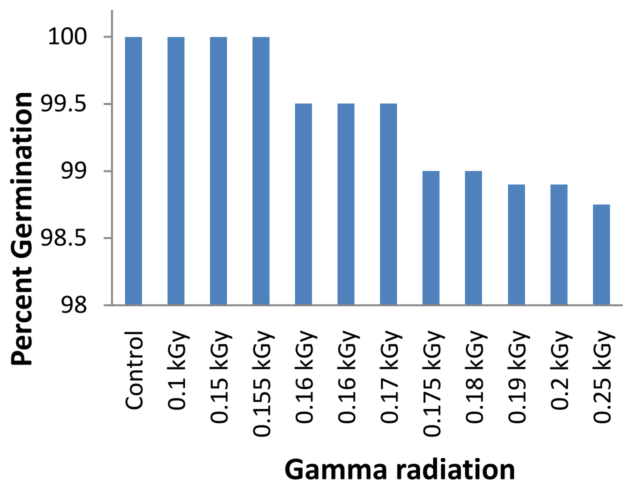

Figure 1: Effect of pre-sowing seed treatment of gamma irradiation on percent germination.

3.1.2 Percent Emergence

According to the results, up to 0.175 kGy dose, gamma irradiation had a significant effect on the emergence percentage after that dose it showed a reduction in the growth attributes. Emergence percentage have found significant at 1% and 5%; CD (0.05) = 15.281 (Table 1).

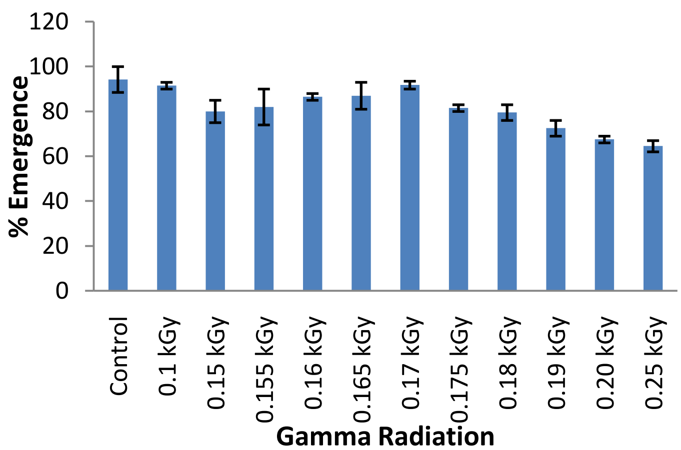

Figure 2: Effect of pre-sowing seed treatment of gamma irradiation on percent emergence

Table 1: Effect of pre-sowing seed treatment of gamma irradiation on percent emergence

Treatments	**Percent Emergence**
Control	94[a]
0.1 kGy	92 [ab]
0.15 kGy	80 [abc]
0.155 kGy	82 [abc]
0.16 kGy	87 [abc]
0.165 kGy	87 [bcd]
0.17 kGy	92 [bcd]
0.175 kGy	82 [bcd]
0.18 kGy	80 [bcde]
0.19 kGy	73 [cde]
0.20 kGy	68 [de]
0.25 kGy	65 [e]
Mean	81.54

3.2 Effect of pre-sowing seed treatment of gamma irradiation on germination raised on Plant height, of wheat.

3.2.1 Plant Height

Plant height indicates significant effect of gamma radiation irrespective of the dose of exposure (Table 2).

Table 2: Effect of different doses of gamma radiation on Plant height of 6, 8, 12 and 14 day old wheat seedlings raised on germination paper at 25 ± 2ºC.

Dose (kGy)	Plant Height (cm)				
	S I	**S II**	**S III**	**S IV**	**Mean**
Control	25.18 ±2.2	31.52 ±6	38.81 ±4.6	35.17±7	32.67[a]
0.1	22.12 ±2.4	28.18 ±6.1	35.1±6	32.82±5.3	29.56[b]
0.15	21.16 ±1.9	25.58 ±5.3	37.1±6.3	34.03±5	29.47[b]
0.155	20.35 ±1.9	26.16 ±5.4	36.46 ±6.9	31.75±4.8	28.68[b]
0.16	21.19 ±1.9	22.15 ±6	38.68 ±3.6	32.08±6.1	28.53[b]
0.165	20.31 ±1.7	23.48 ±4	36.6±7.1	35.27±5.3	28.92[b]
0.17	19.23 ±1.8	24.53 ±5.2	36.94 ±6.6	33.14±5.8	28.46[b]
0.175	19.74 ±1.7	23.35 ±5	36.35 ±3.7	33.93±4.2	28.34[b]
0.18	18.93 ±2.3	23.86 ±3.1	36.67 ±4.4	32.12±7.7	27.90[b]
0.19	17.28 ±1.6	21.32 ±3.6	31.06 ±3.2	31.41±5.2	25.27[c]
0.20	14.25 ±1.9	19.3±3.5	27.82 ±5.4	29.3 ±5.7	22.67[d]
0.25	12.94 ±1.7	16.17 ±2.5	27.15 ±2.6	25.58±5.9	20.46[e]
Mean	19.39	23.80	34.90	32.22	
CD (0.05) = 2.172					

Mean with the same letter are not significantly different at p<0.5.

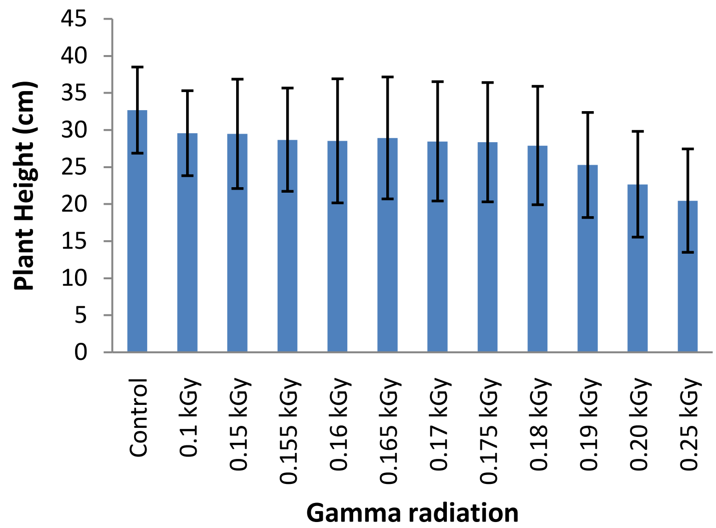

Figure 3: Effect of gamma radiation on plant height of seeding raised on germination.

a) Effect of gamma radiation of plant height on field

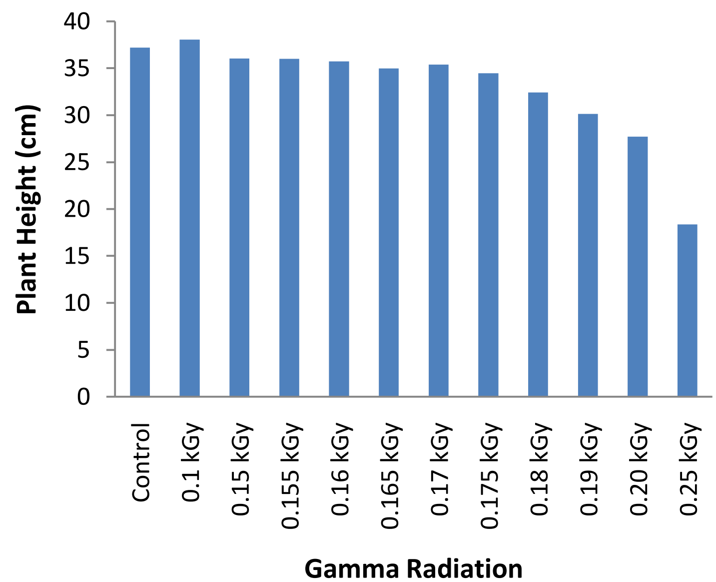

Figure 4: Effect of pre-sowing seed treatment of gamma irradiation on plant height (cm)

Plant height was found to be significant at 5% of CD i.e., 8.263. Maximum value was found to be at 0.1 kGy (38.06) as compared to the control and the other irradiated treatments.

Table 3: Effect of pre-sowing seed treatment of gamma irradiation on plant height

Dose (kGy)	Plant Height (cm)		Mean
	S I	S II	
Control	16.65±0.99	57.73 ±8.55	37.19[a]
0.1	20.23±7.06	55.88±7.80	38.06 [a]
0.15	16.33±5.81	55.73±7.05	36.03 [a]
0.155	17.08±5.52	54.92±8.76	36.00 [a]
0.16	16.82±3.97	54.67±6.52	35.74 [a]
0.165	16.08±6.71	53.85±8.74	34.97 [ab]
0.17	16.92±0.81	53.90±6.12	35.41 [ab]
0.175	15.78±2.68	53.15±8.19	34.47 [ab]
0.18	14.00±4.20	50.83±4.27	32.42 [ab]
0.19	12.23±2.37	48.02±7.78	30.12 [ab]
0.20	9.68±1.85	45.72±7.20	27.70 [b]
0.25	7.87±2.65	28.88±14.91	18.37[c]
Mean	14.974	51.107	
CD(0.05) = 8.263			

Mean with the same letter are not significantly different at $p<0.05$

3.2.2 Plant biomass

Plant mass, in general was constituted by shoot at large with very little contribution from roots. Plant biomass increased at 0.18 kGy and then started to decrease. Non-radiated seed i.e. control have the maximum plant biomass. Plant biomass found significant results under laboratory condition as compared to the field conditions (figure 5).

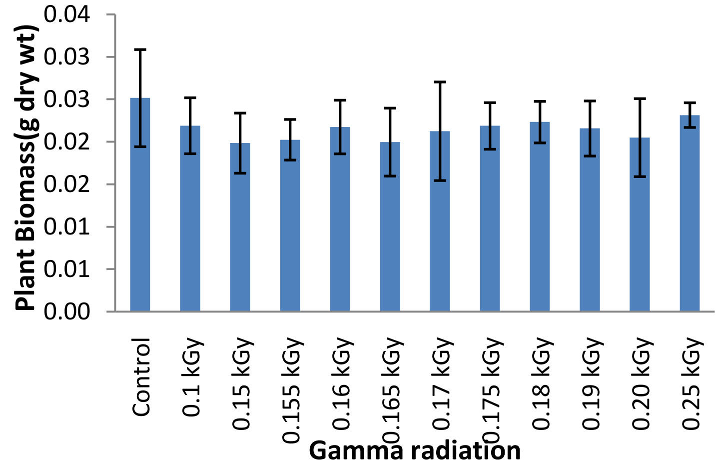

Figure 5: Effect of gamma radiation on plant biomass of seeding raised under germination paper

a) The results showed that the maximum average mean value of plant biomass of irradiated seeds was found at 0.1 kGy (0.581) and minimum average mean value was found at 0.25 kGy (0.065). Treatment of irradiated seeds of plant biomass was found to be non significant.

Table 4: Effect of pre-sowing seed treatment of gamma irradiation on plant biomass

Dose (kGy)	Plant Biomass (g dw)			Mean
	S I	S II	S III	
Control	0.036	0.153	1.726	0.638
0.1	0.034	0.128	1.581	0.581
0.15	0.029	0.134	1.504	0.556
0.155	0.028	0.117	1.259	0.468
0.16	0.028	0.110	1.209	0.449
0.165	0.026	0.110	1.067	0.401
0.17	0.022	0.087	1.032	0.380
0.175	0.019	0.096	1.055	0.390
0.18	0.018	0.081	0.955	0.351
0.19	0.015	0.047	0.503	0.188
0.20	0.010	0.021	0.230	0.087
0.25	0.005	0.015	0.174	0.065

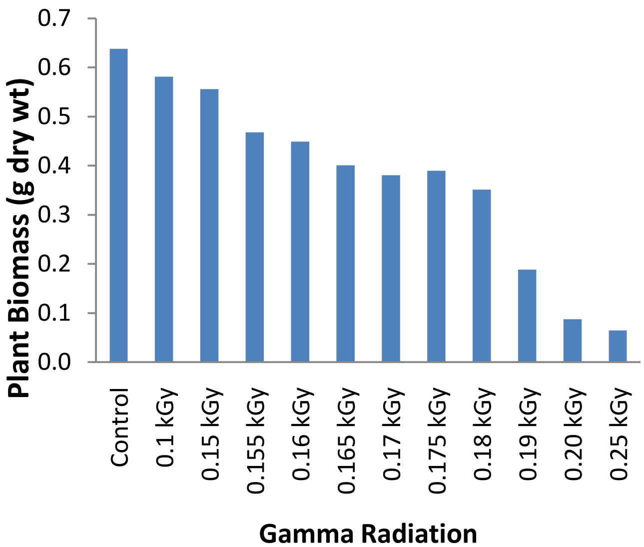

Figure 6: Effect of pre-sowing seed treatment of gamma irradiation on plant biomass (g dw)

3.2.3 Seedling Vigour Index I
Plant Vigour was shown dose-dependent response (Table 5). Maximum Vigour Index was found at 0.175 kGy, beyond this limit it started decreasing.

3.2.4 Seedling Vigour Index II
The capacity for natural growth and survival, as of plants or animals. Table 6 show the effect of gamma irradiation on strength of seedling raised under germination paper.

Table 5: Effect of different doses of gamma radiation on Plant vigour index I of 6, 8, 12 and 14 day old wheat seedlings raised on germination paper at 25 ± 2ºC

Dose (kGy)	Vigour Index I				Mean
	S I	S II	S III	S IV	
Control	2455.1	3073.2	3784.0	3429.1	3185.3^{a}
0.1	2184.4	2782.8	3466.1	3241.0	2918.6^{b}
0.15	2116.0	2558.0	3710.0	3403.0	2946.8^{b}
0.155	2035.0	2616.0	3646.0	3175.0	2868.0^{b}
0.16	2092.5	2187.3	3819.7	3167.9	2816.8^{b}
0.165	2031.0	2348.0	3660.0	3527.0	2891.5^{b}
0.17	1899.0	2422.3	3647.8	3272.6	2810.4^{b}
0.175	1974.0	2335.0	3635.0	3393.0	2834.3^{b}
0.18	1893.0	2386.0	3667.0	3212.0	2789.5^{b}
0.19	1728.0	2132.0	3106.0	3141.0	2526.8^{c}
0.20	1425.0	1930.0	2782.0	2930.0	2266.8^{d}
0.25	1277.8	1596.8	2681.1	2526.0	2020.4^{e}
Mean	1925.9	2364.0	3467.1	3201.5	
CD (0.05) = 217.803					

Mean with the same letter are not significantly different at $p<0.05$

Table 6: Effect of different doses of gamma radiation on Plant vigour index II of 6, 8, 12 and 14 day old wheat seedlings raised on germination paper at 25 ± 2ºC

Dose (kGy)	Vigour index II				Mean
	S I	S II	S III	S IV	
Control	3.22	2.34	2.24	1.95	2.44
0.1	2.57	2.37	1.98	1.78	2.17
0.15	2.30	1.60	2.30	1.70	1.98
0.155	2.00	2.40	1.80	2.00	2.05
0.16	2.37	2.47	1.98	1.78	2.15
0.165	2.30	2.30	1.90	1.50	2.00
0.17	2.67	2.37	2.07	1.28	2.10
0.175	2.30	2.50	1.90	2.00	2.18
0.18	2.60	2.20	2.10	2.00	2.23
0.19	2.20	2.60	1.80	2.10	2.18
0.20	2.70	2.10	1.80	1.60	2.05
0.25	2.47	2.27	2.17	2.17	2.27
Mean	2.47	2.29	2.00	1.82	
CD (0.05) = NS					

4. DISCUSSION
Gamma irradiation is a reliable and safe method for improving the shelf life and nutritional quality of the stored food. Recently, low doses of gamma irradiation were shown to improve plant vigour and yield of wheat. It is logical to imply that the induced stimulation of plant growth is a consequence of gamma ray affected changes in biochemical characteristics of target tissue or plant which directly or indirectly regulate cell division and cell elongation

and thus the morphological aspects and consequently the related physiological attributes. Gamma radiation had no significant effect on final germination percentage. Irradiated wheat seeds kept their germination capacity compared to the control. Maximum decrease in germination percentage/percentage emergence was observed with 0.25 kGy. These results were in accordance with the germination test done by Melki & Marouani (2009) whereby there was no significant difference in germination. Gamma ray imposed a significant impact on plant height. The highest length of plant (38.06, 32.6) was observed at 0.1 kGy and control respectively, under field and laboratory conditions. By increasing radiation dose, plant height declined compared after 0.1 kGy dose. Maximum reduction in plant length was observed at 0.25 kGy. The symptoms frequently observed in the low-or high-dose irradiated plants are enhancement or inhibition of germination, seedling growth, and other biological responses (kim *et al.,* 2000; Wi *et al*., 2007). Although, no certain explanation for the stimulatory effects of low dose gamma radiation are available until now, in accordance to the results obtained by Wi et al., (2007), there is a hypothesis that low dose radiation will induce the growth stimulation by changing the hormonal signalling network in plant cells or by increasing the anti oxidative capacity of the cells to easily overcome daily stress factors such as fluctuations of light intensity and temperature in the growth conditions (Wi et al., 2007). Results showed a significantly lower plant weight compared to the control under both field as well as laboratory conditions. Maximum reduction in plant weight was observed after 0.18 kGy. Plant mass, in general, was constituted by shoot mass at large with very little contribution from roots. Shakoor et al., (1978) and Khalil et al., (1986) attributed decreased shoot lengths at higher doses of gamma rays to reduced mitotic activity in meristematic tissues and reduced moisture contents in seeds respectively. Mean plant mass measured at three stages of growth. Plant mass increased significantly only at 175 Gy radiation dose. Plant biomass more or less followed a similar pattern of dose-dependent response to ionizing radiation. Minimum plant dry weight was observed at 0.25 kGy in HD2967. The reduction in both plant length and plant biomass is dependent upon the radiation dose. However, Wi et al., (2007) reported no significant morphological aberration in the phenotype of plants irradiated with relatively low doses of gamma rays, while high-dose irradiation inhibited seedling growth remarkably. Radiation processing of food results in enhancing food conservation, improvement of food hygiene and overcoming quarantine barriers of food exporters. Radiation processing of food can therefore be applied for strengthening food security, for improving food safety and for increasing international trade. It can improve the food security by cutting down food losses caused by storage conditions, insects, microorganism and physiological changes during storage and transportation. A dose of radiation between 1 and 10 kGy is generally used in the reduction of microbial load and elimination of pathogenic microorganism or for the extended shelf life; whereas a dose of below 1 kGy is used for insect disinfestations, delayed senescence and ripening, and sprout inhibition. Today, internationally 10 countries worldwide including India have approved radiation processing of more than 400 food items for various purposes. In India, PFA has so far approved 15 food products. An immense potential still exists for further inclusion of various irradiated food product in the PFA approval list. The radiation process is approved under international regulation bodies including WHO, FAO and Codex. Efforts will have to be made to expedite the inclusion of various fruits and vegetables in the list of products approved for radiation processing. Besides this, awareness among the consumers and regulatory agencies about the advantage of radiation processing has to be brought in. Once this is done the radiation process in fruits should set up in places closer to the source of the horticultural produces.

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