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Beta Radiation Effects on the Biochemical and Morphological Properties of *Phaseolus vulgaris*

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ABSTRACT:

This paper presents the investigation of the effects of beta radiation on common bean (Phaseolus vulgaris). The seeds are irradiated with Sr-90 source in the range of 0.36 to 2.2 mrad. The LD_{50} is found to be 1.0902 mrad. Increase in germination percentage and speed is observed under different radiation doses. The total chlorophyll content is found higher in irradiated plantlets than the non irradiated ones. The highest chlorophyll content is found in the dose of 0.3634 mrad, which also shows higher shoot height. The chlorophyll a is found to be higher than chlorophyll b. However the chlorophyll ratio is found to be lower in all radiated plantlets compared to non radiated ones. So it can be concluded that low dose of beta radiation is found to have stimulating effects on the growth of the plantlets, maximum growth is observed at 0.3634 mrad. However higher doses have a deteriorating effect on plant growth.

Key Words: beta radiation, *Phaseolus vulgaris*, chlorophyll content, plantlets, LD_{50}

INTRODUCTION

The study of the effects of ionizing radiation on plants is a broad, diverse and complex field of work. Several works has been conducted on the effects and influence of these radiations on diverse varieties of plants. Ionizing radiation is reported to influence the plant by inducing changes in certain biological, cytological, genetical, physiological and morphological processes (Gunckel and Sparrow, 1961; Thapa, 2004). Ionizing radiations has been widely applied in modern agricultural methods as a tool for improving genetical diversity and crop improvements. They are used to sterilize crops to reduce pathogen attack, increase viability, while in others stimulate germination. Radiation results changes in both physiological and biochemical processes. It causes changes protein synthesis, hormone, enzyme activities and photosynthesis (Xiucher, 1994; Rabie *et.al.*, 1996; Stoeva and Bineva, 2001). The effects are studied over in large number of plants including both gymnosperms and angiosperms. The studies has shown that the higher radiation doses are usually found to be inhibitory (Kumari and Singh, 1996; Radhadevi and Nayer, 1996; Rabie *et.al.*, 1996; Marcu *et.al.*, 2013) whereas the lower doses are found to be stimulatory sometimes (Chicea and Racuciu, 2007; 2008, Bhattacharya *et.al.*, 2010; 2012; Grover, 2014; Roy, 2015).

In this investigation the effect of beta radiation on common bean is studied. A common bean or Kidney bean (*Phaseolus vulgaris)* which is under the family fabaceae is commonly growing vegetable crop in tropical countries and a huge source of dietary proteins. The nutritional value of kidney bean is quite high. The 100g contain 130 kJ energy, 7 g carbohydrates, 1.4 g sugars, 3.4 g dietary fibre, 0.1 g fat, 1.8 g protein, 35 μg vitamin A, 16 mg vitamin C, 37 mg calcium (Choung *et.al.*, 2003). The main purpose of the study is to observe the beta radiation effects on the biochemical and morphological parameters of this crop.

MATERIALS AND METHODS

Seeds of pure strain of common bean (*Phaseolus vulgaris*) are used in this study. The surface sterilized seeds were soaked in distilled water overnight and then subjected to irradiation treatment of doses ranging between 0.36 to 2.2 mrad. A special irradiation chamber was designed for this purpose (Fig.1).

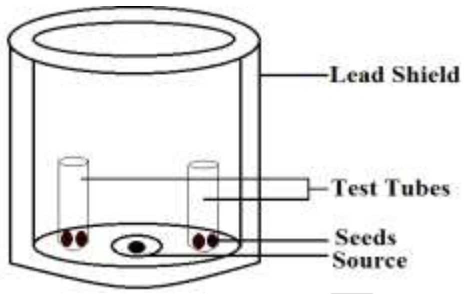

Fig.1. Beta irradiation chamber

Batches of 20 seeds were irradiated at a time. The ß source utilized is Sr-90 of Beta energy ($E_ß$) -2.274, Activity - 0.05 µCi and Half life 28.79 years.
The decay chain of Sr-90 is as follows:

$$^{90}Sr \xrightarrow{-ß} {}^{90}Y \xrightarrow{-ß} {}^{90}Zr$$

Seed Viability Test

Seed viability test is conducted, which is based on the rate of germination, to detect delay in germination and other physiological problems if any associated with the seeds. The emergence of radicle is taken as an indicator of germination. The seeds were first surface sterilized by soaking in 0.1 % mercuric chloride for 1 min, followed by washing thrice in distilled water. The seeds were then allowed to germinate in moist filter paper with humidified cotton. The germination rate is measured on 4^{th} day. The seeds are found to be 90% viable.

Germination Speed, Percentage and Water Imbibition Percentage

The germination speed is determined using the following formula (Chiapusio *et.al.*, 1997):
S (seedday^{-1}) = (N_1 X 1) + (N_2- N_1) X ½ + ………………….(N_n – N_{n-1}) X 1/n.
Where N1, N2,……….., N_{n-1}, N_n = Proportions of germinated seeds observed at 1, 2,……..n-1 and n days.

The germination percentage is measured on 4th day using the formula:

$$\text{Germination Percentage} = \frac{\text{Total number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Water imbition percentage is calculated as Wi = (Wi - Wd) / Wd X 100
Where, Wi and Wd are the weights of imbibed and dry seeds respectively. Imbibition was measured after 24 hrs of soaking in water.

Radiation Sensitivity Detection

Radiation sensitivity test were conducted based on height increment of the plantlets. The LD_{50} (LD=Lethal dose) is used to determine the beta dose which results in half of the height of plantlets.

Measurement of Radicle and Shoot Length

The seeds were allowed to germinate in humidified petridishes. The radicle lengths are measured regularly from the time of emergence of radicles with 1.0 mm precision every day. Successively plastic pots of size (21 X 20 X 15) cm were filled with homogeneous garden soil. Seeds are then soaked in the soil and all pots are watered up to saturation and kept in open and were irrigated regularly. The shoot lengths were measured at an interval of 5 days up to the period of 30 days.

Biochemical Assays

Different biochemical estimates are conducted to investigate differential effects of beta radiation. The carbohydrate and protein estimation are done by Anthrone (Hedge and Hofreiter, 1962) and Lowry's method (Lowry *et.al.*, 1951) respectively. Enzyme assay of amylase (Bernfeld, 1955) and catalase activity (Aebi, 1983) is determined. Assimilatory pigment level (chlorophyll a, chlorophyll b and total chlorophyll) is determined according to Arnon (Arnon, 1949).

Statistical Analysis

This study is repeated thrice and in three replicates. The results were statistically analyzed using ANOVA and Tukey's test of significance.

RESULTS AND DISCUSSION

Radiation Sensitivity Detection

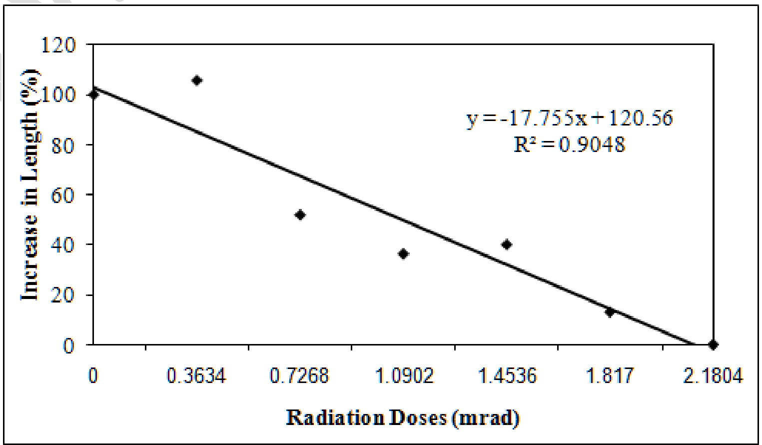

***Fig.2. The radiation sensitivity detection*.**

The radiation sensitivity of *Phaseolus vulgaris* is determined by measuring the height increment of 5 day irradiated plantlets compared with the non irradiated ones. The present data indicates the stimulation in plant growth upto 0.3634 mrad and then inhibition occurs with higher doses. The LD_{50} (Lethal dose that killed 50% of the plantlets) found is 1.0902 mrad (Fig. 2). This suggests the increased sensitivity of radiation to higher doses; this may be the effect of reduced secretion of growth regulators.

Germination Speed and Germination Percentage

The germination speed, germination percentage and water imbibition percentage is determined to observe the effects of radiation on seed germination properties (Table.1). The germination speed and germination percentage are found to be slightly affected with increase in radiation dose. The germination percentage decreases successively with increase in dose. Similar results are also observed for germination speed. The highest germination is recorded in dose of 0.3634 mrad. The dose of 2.1804 mrad brings the germination to about half that of the non irradiated ones. Therefore the dose of 2.1804 mrad can be predicted to have lethal effects on germination properties. The water imbibition percentage is observed to show minute variations in radiated ones.

Table1. Mean, standard deviation, germination percentage (G%), germination speed (GS) and water imbibition percentage (WI%) of radicle lengths (cm)

Doses (mrad)	Mean	S.D	G%	GS	WI%
0	2.01^{a}	0.2059	60	6.5	49.99281
0.3634	1.42^{b}	0.2337	90	11	54.83871
0.7268	1.24^{bc}	0.3373	80	8.166667	52.19009
1.0902	1.07^{c}	0.3553	85	8.166667	55.65091
1.4536	0.75^{d}	0.1814	80	8.5	52.8447
1.817	0.46^{e}	0.1188	75	7.75	52.71779
2.1804	0.36^{e}	0.1191	35	3.666667	52.10267

G% = Germination Percentage, GS = Germination Speed, WI % = Water imbibitions Percentage

Means with different letters are statistically different between treatments by tukey test (p = 0.05)

Radicle Lengths and Shoot Lengths

The radicle length shows an inverse relationship with the increase in dose (Fig. 3). All irradiated samples are found shorter in radicle length when compared to the non irradiated ones.

The variations in height (shoot length) of radiated and non irradiated plantlets are presented in Table. 2. The results show the highest shoot growth at a dose of 0.3634 mrad. The further increase in dose has a negative effect on shoot length. The shoot lengths decrease with increase in dose after 0.3634 mrad dose. Many researchers have reported such a stimulated low dose exposure in several different species (Charbaji and Nabulsi, 1999; Klarizze, 2005). The reasons of these stimulations may be attributed to the accelerated cell division or activation of growth regulators.

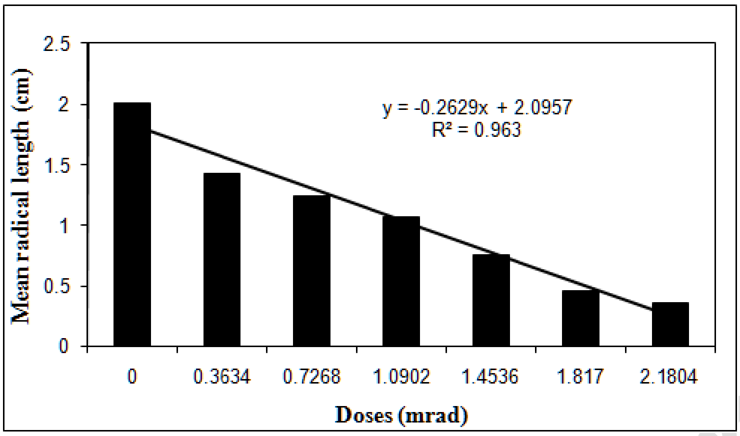

Fig.3. Mean radicle lengths of the germinating seeds

Table 2. The height of plantlets (cm) upto 30 days of growth at an interval of 5 days.

Doses (mrad)	5DAP	10DAP	15DAP	20DAP	25DAP	30DAP
0	6.656[a]	13.312[b]	18.432[b]	23.04[b]	42.24[b]	57.088[b]
0.3634	5.12[b]	13.824[a]	25.088[a]	39.424[a]	54.528[a]	72.704[a]
0.7268	3.84[c]	5.12[c]	7.424[c]	10.24[c]	12.8[c]	12.8[c]
1.0902	2.816[d]	4.864[cd]	7.168[c]	7.68[d]	10.24[d]	10.496[d]
1.4536	2.56[d]	4.864[d]	5.632[d]	5.632[e]	6.4[e]	7.68[e]
1.817	1.28[e]	2.304[e]	3.072[e]	3.072[f]	3.84[f]	5.12[f]

Means with different letters are statistically different between treatments by tukey test (p=0.05) (DAP =Days after plantation)

Biochemical Assays

In both radiated and non radiated samples no such differentiation can be done based on the biochemical contents. The protein and carbohydrate contents were found to show very little deviation based on the dose.

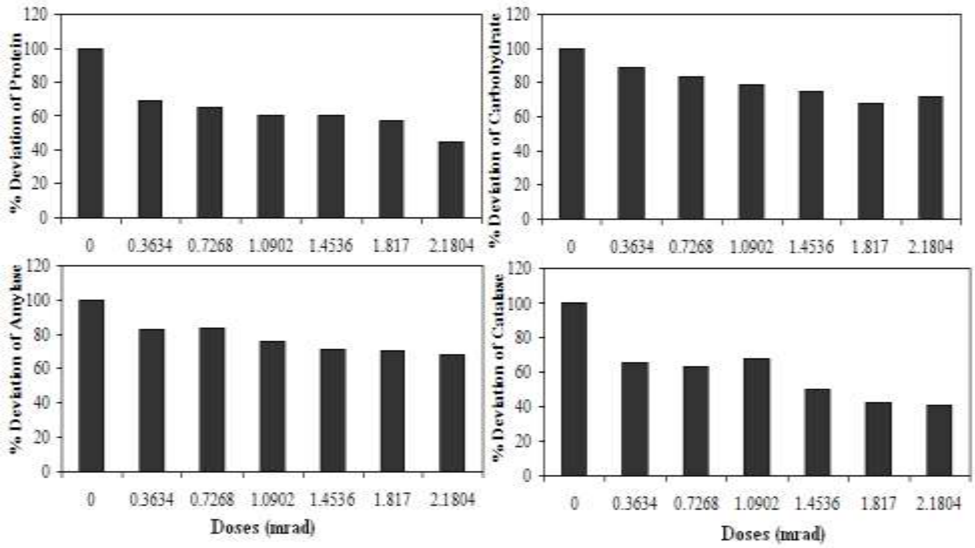

Fig. 4. Percentage deviation in protein, carbohydrate, amylase and catalase contents

Similar results were also obtained from enzyme activity of the amylase and catalase (Fig. 4). However all the results are found to deviate negatively when compared to the non irradiated ones. Therefore the study showed an irregular distribution of biochemical estimates. Radiation stress in plants results in developing certain defense mechanisms, which causes changes in biochemical parameters. Radiation is known to produce oxidative stress with the production of reactive oxygen species. These are found to increase the activity of antioxidants. Several enzymes are reported to maintain the defense against radiation stress. Catalase is known to destroy the H_2O_2 produced by several reactions (Singh *et.al.*, 1993; Cho *et.al.*, 2000).

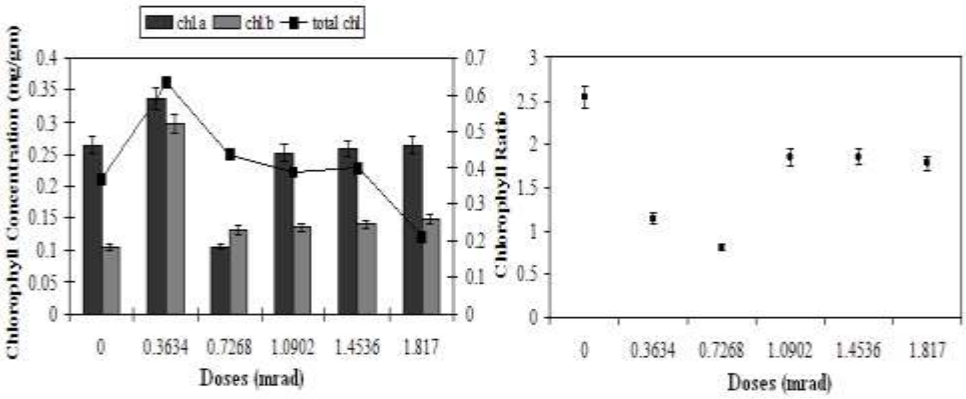

Fig. 5. Chlorophyll content and chlorophyll ratio of the plantlets

The chlorophyll content is determined after 30 days of plantation (DAP). The total chlorophyll content is found higher in irradiated plantlets than non-irradiated ones except in the dose of 1.817 mrad. The highest chlorophyll content is found in the dose of 0.3634 mrad. The chlorophyll ratio is however found to be lower in all radiated plantlets compared to non radiated plantlets (Fig. 5). Chlorophyll content is found to be sensitive to lower doses of radiation. The chlorophyll a is found to be higher than chlorophyll b in all plantlets, with the single exception of 0.7268 mrad where chlorophyll a concentration is slightly less.

CONCLUSIONS

Increasing beta dose rate increases the plant height, germination percentage, germination speed and chlorophyll content up to a dose of 0.3634 mrad. However with the further increase in dose all the parameter decreases. LD_{50} is found to be 1.0902 mrad. No biochemical differentiation is found in biochemical properties of irradiates plantlets based on dose variations. In general it can be concluded that the higher radiation doses had negative effects on the kidney bean seedlings. More research is required to investigate on the stimulatory effects of beta radiation on plant growth.

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