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EFFECT OF GAMMA RADIATION ON GERMINATION AND PHYSIOLOGICAL ASPECTS OF PIGEON PEA (*CAJANUS CAJAN* (L.) MILLSP). SEEDLINGS

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ABSTRACT

Gamma irradiation is one of the physical mutagen that widely used for mutation breeding, food sterilization and medicinal healing. In the present study irradiation techniques were applied to investigate the effect of gamma irradiation on germination and physiological aspects of pigeon pea seedlings. Pigeon pea (Var. BSMR 736) seeds were irradiated with gamma rays (5,10,15,20,25Kr). The results shown that the germination frequency, shoot and root length decreased with increasing radiation doses. Germination frequency was high (95.89) in control plants and low (66.09) in 25kr irradiated plantlets. Total protein content was high in plantlet irradiated with 5kr (12.60mg/g FW) where as only 9.21mg/gFW) was found in control plants. Proline content was high in 25Kr plantlets (9.93µmoles/g FW) less in 10Kr irradiated plantlets. Highest amount of chlorophyll was found in 25Kr irradiated plantlets (3.84mg/gFW) and least (2.18mg/gFW) was found in 15Kr irradiated plants. In addition the amount of chlorophyll a was higher than chlorophyll b in both irradiated and non-irradiated plantlets.

KEYWORDS: *Cajanus cajan*, Gamma Irradiation, in Vitro Mutagenesis

INTRODUCTION

In the last decade, gamma irradiation has been drawn the attention as a new and rapid method to improve the qualitative and quantitative characters of many crops. Gamma irradiation has been widely applied in medicine and biological effects induced by a counter intuitive switch -over from low doses stimulation to high doses inhibition (Charbaji and Nabulsi, 1995). Previous studies have shown relatively low doses ionising radiation on plants and microorganisms are manifested as accelerated cell proliferation, germination rate, cell growth, enzyme activity, stress resistance and crop yields (Chakravarthy and Sen, 2001). Inhibition of seed germination, shoot and root elongation have been reported for detection of irradiated cereal grains and legumes. Chaudhuri (2002) reported that the irradiation of wheat seeds reduced shoot and root lengths upon germination. Gamma radiation can be useful for the alteration of physiological characters (Kiong *et al* 2008). The biological effect of gamma rays is based on the interaction with atoms or molecules in the cell, particularly water, to produce free radicals (Kova'cs and Keresztes 2002.)

In vitro mutagenesis provides an opportunity to increase variability of an economically important cultivar used on plants in developing varieties that are agriculturally and have high productivity potential (Jain *et al* 1998). Traits induced by mutagenesis include plant size, blooming time and fruit ripening, fruit colours, self compatibility, resistance to pathogens and also it is known to increase nutritive values of food sources and also enhance, accelerate growth of certain vegetables (Predieri, *et al*, 2001). Gamma rays are known to influence plant growth and development by

influencing cytological, morphogenetic changes in cells and tissue Genkel and Sparrow, 1961). Mahamune *et.al*. (2011) reported induction of morphological mutants in French been by using gamma rays and Sodium azide. Giri. *et.al* (2010) reported high yielding mutants of pigeon pea by using EMS. The objective of present study is to study the effect of gamma irradiation on pigeon pea seedlings.

MATERIALS AND METHODS

Seeds of Pigeon pea (var. BSMR 736) were obtained from agricultural research station Gulbarga. Seeds were subjected to irradiation by gamma rays at BARC Mumbai. Different doses (5,10,15,20,25Kr) were given to the pigeon pea seeds and they were allowed to germinate on filter bridge containing 5ml of distilled water. Seeds were allowed to germinate and number of germination frequency was calculated. After two weeks shoot and root length were recorded.

Determination of Total Soluble Protein

Total protein content of the irradiated and non irradiated plantlets was determined using the Lowry's method (Lowry *et al* 1951) 500mg of leaf sample was grinded well in 10ml of phosphate buffer (0.2M:PH 7.0) with a Pestle and mortar. Extract was centrifuged at 5000xg for 15 minutes. Supernatant was used for estimation. 0.1ml of the extract was taken in test tube. 1ml of alkaline copper reagent was added. Stirred gently and allowed to stay at room temperature for 15 min. 0.2ml of diluted 1:1 folin – ciocalteau- Phenol reagent was added. After 30 min of incubation at room temperature for 15 min. After 30 min of incubation at room temperature in dark, blue colour was develops. Volume was made to 5ml with double distilled water. The absorbance was red at 500nm wavelength.

Determination of Chlorophyll Content

Chlorophyll content of non irradiated and irradiated plantlets was determined using Acetone method (Arnon, 1949). 200mg of freshly cut leaves were grinded in 10ml of 80% acetone. The homogenate was filtered in a volumetric flask (25ml). Final volume of the filtrate was made up to 25ml with 80% acetone. Absorbance was recorded at 663 and 645nm separately. The amount of Chlorophyll was calculated. The Chlorophyll content in milligram per litre was determined according to the formulae given below and further expressed in milligram per gram fresh weight of plant material.

Chlorophyll a = $(2.7xA_{663})$- $(2.69xA_{645})$ xv/1000xw

Chlorophyll b = $(22.9xA_{645})$-$94.68xA_{663})$ xv/1000xw

Total Chlorophyll = $(8.02xA_{663})$ + $(20.2xA_{645})$xv/1000xw

Determination of Proline

Proline content of non irradiated and irradiated plantlets was determined by the method adopted by Bates *et.al* (1973). 0.5g of flesh leaf sample was homogenized in 10ml of 3 percent aqueous sulphosalicylic acid and the homogenate was filtered using what man's No 1 filter paper. Two ml filtered was taken in a test tube and 2ml of acid ninhydrin and 2ml of glacial acetic acid was added. This was allowed to react for 1 hour at 100^0c in a boiling water bath. The reaction was terminated by placing the tube in an ice box. 6ml of toluene was added to the reaction mixture. The chromophore containing toluene was separated and absorbency was recorded at 520nm wavelength using toluene as blank.

Statistical Analysis

The experimental design was completely randomized blocks. The analysis of variance (ANOVA) was used to determine the differences in average of all tested parameters between irradiated and non irradiated plantlets.

RESULTS AND DISCUSSIONS

Germination and Growth

Table 1: Effect of Gamma Radiation on Germination, Shoot Length, Root Length and Number of Lateral Roots of *Cajanus cajan* (L) Millsp. Seedlings

Radiation Doses	Germination Frequency	Shoot Length	Root Length	Total Seedling Length	No. of Lateral Roots
0Kr	95.89±0.20	7.68±0.58	13.12±0.58	14.28±0.84	13.49±0.70
5Kr	95.83±0.7	7.65±0.49	9.94±0.65	11.76±0.95	9.43±1.37*
10Kr	91.80±0.57	7.60±0.42	9.39±0.58	11.41±0.55	8.56±0.73
15Kr	75.81±0.40	7.40±0.13	3.73±0.58*	10.01±0.55	8.44±0.77
20Kr	70.88±0.73	5.52±0.13	3.92±0.67	7.32±0.36	7.22±0.89
25Kr	67.09+09	5.13+0.37	2.41+0.41*	6.79+0.55	7.16+0.78

* P>0.05, ** P<0.01, *** P<0.05

Gamma irradiation had some effect on germination frequency of irradiated pigeon pea seeds. Germination frequency was decreased significantly after higher irradiation doses ranging from 15Kr to 25Kr. Germination frequency was not much affected to seeds irradiated with 5kr and control. Highest germination percentage was observed in control plants (Table 1) (95.83). Maximum decrease in percentage was observed in 25Kr irradiated plantlets (67.09). These records were in accordance with the result obtained by Amjad *et al* (2008) with chickpea. The results of Kiong *et al* (2008) shown that survival of plants to maturity depends on the damage nature and extent of chromosomal damage. Increasing frequency of chromosomal damage with increasing radiation dose may be responsible for less germination and reduction in plant growth and survival. Changes in the germination percentage were found to attribute to gamma rays treatments. The stimulating causes of gamma ray on germination may be certified to the activation of RNA or protein synthesis, which occurred during the early stage of germination after seed irradiated (Abdel-Hady *et.al*., 2008). Shoot length decreased in all doses of irradiation as compared to non irradiated. Maximum decrease was observed after 20kr dose (Amjad *et.al* 2008). In the present study, the variability as measured by mean values of the root/shoot lengths decreased with increase in the radiation dose. Choudhari (2002) reported that when radiation is sufficient to reduce the rooting percentages, then the root lengths do not exceed a few millimetres in length. Due to metabolic disorders in the seeds after gamma irradiation, the seeds are unable to germinate.

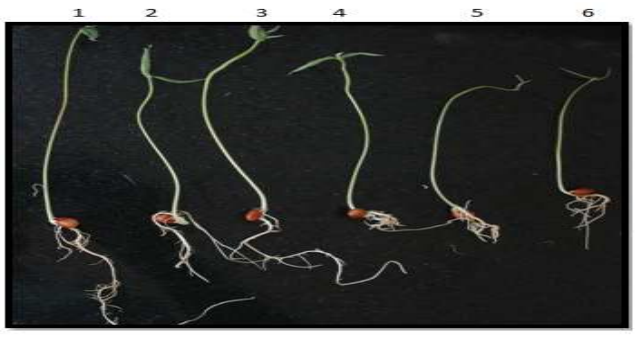

Figure 1: Effect of Radiation on Seedling Length, Root Length and Number of Roots of *Cajanus cajan* (L) Millsp. Seedlings (Lane 1,2,3,4,5,6 Indicates Control, 5Kr, 10Kr15Kr,20Kr,25Kr Radiation Doses Respectively)

Table 2: Effect of Gamma Radiation on Protien and Proline Content on *Cajanus cajan* (L.) Millsp. Seedlings

Radiation Doses	Protein Content (Mg $G^{-1}FW$)	Proline (µmole $G^{-1}FW$)
0Kr	9.21±0.73	8.9±0.91
5Kr	12.60±1.17	8.76±0.90
10Kr	12.42±1.44	4.38±1.12
15kr	11.35±0.71	9.88±1.27
20Kr	12.53±2.18	9.61±1.02
25Kr	11.92±1.80	9.93±1.4

* P>0.05, ** P<0.01, *** P<0.05

Determination of Total Soluble Protein

Total soluble content protein content of the irradiated pigeon pea seeds shown some differences depending on the gamma irradiated doses. Biochemical differentiation based on the total soluble protein content revealed that plantlet irradiated at 5Kr contain highest amount of total soluble protein (12.60mg/gFW), whereas only (9.21mg/gFW) was observed in control plants (Table 2). All the irradiated plantlets shown higher level of proteins when compared to control plantlets. In this study it was found that there was an irregular distribution of total soluble protein content in both irradiated and non irradiated seedlings. Increase in protein content of mutants observed in present studies is in agreement with the results obtained by Jijiya (1986), and Sudharani (1990). Anna Kiong reported irregular distribution of protein in irradiated as well as non irradiated plantlets of *Citrus sinensis***.** The results are in agreement with the reports made by Anna Kiong.

Humera (2006) stated that the stress reaction of plants often results in the alteration of protein metabolism. Several proteins were synthesized and accumulated plant tissues under a range of stress condition such proteins are referred to as stress factors. The most crucial function of plant cell is to respond to gamma stress by developing defence mechanisms. This defence was brought by alteration in the pattern of gene expression altered under gamma stress, qualitative and quantitative changes in total soluble protein content was obvious (Corthals *et.al*. 2000). These proteins might play a role in signal transduction, anti-pathogenesis or osmolyte synthesis which were essential to plants function and growth Zolla, *et.al*., 2003).

Effect of Gamma Radiation on Proline Content

Biochemical differentiation based on proline content revealed that plantelets irradiated at 0,5,10,15,20,25Kr exhibited proline content of 8.9µmoles/gFW, 8.76µmoles/gFW, 4.1µmoles/gFW, 09.34µmoles/gFW, 4.15µmoles/gFW and 9.93µmoles/gFW respectively (table 2). Control plants were showing highest amount of proline content. However there was no significant difference among the plantlets irradiated with 15 and 20Kr. 5 and 25Kr plantlets have shown maximum amount of 1.65 and1.67mg/g Fw.

Gamma irradiation was reported to induce oxidative stress with overproduction of reactive Oxygen species (ROS) such as superoxide radicals, which react rapidly with almost all structural and functional organic molecules, To avoid oxidative damage, plants have evolved various protective mechanisms to species in-cellular compartments (Kiong *et.al*, 2008). This defence was brought about by alteration in the pattern of gene expression (Borzouei *et.al* 2008). This led to modulation of certain metabolic and defensive pathways. one of the protective mechanisms in the synthesis of osmolytes which is essential to plant growth was proline synthesis (Esfandiari *et al.* 2008).

Table 3: Effect of Gamma Radiation on Chlorophyll Content of *Cajanus cajan* (L.) Millsp. Seedlings

Radiation Doses	Chl A	Chl B	Total Chl
Control	1.423±0.54	0.9900±0.67	2.420±1.43 *
5Kr	1.843±0.58 *	0.9200±0.49 *	2.500±0.64*
10Kr	2.307±0.98*	0.6083±0.69*	3.097±1.21**
15kr	2.617±1.07***	0.6787±0.27*	2.487±1.08*
20Kr	2.313±1.34*	0.9467±0.77*	2.877±1.47***
25Kr	2.075±0.95*	1.5±0.90***	3.15±1.86 **

* P>0.05, ** P<0.01, *** P<0.05

Effect of Gamma Radiation on Chlorophyll Content

The results shown that (table 3) irradiated seedlings to 25Kr (3.84mg/gFW) has maximum amount of chlorophyll content compared to rest. Irradiated seedlings shown high amount of chlorophyll content when compared to non irradiated. Chlorophyll a was higher than chlorophyll b in both irradiated as well as non irradiated. In the present study, the chlorophyll content shown irregular distribution among the irradiated seedlings. This result was supported by Kim *et al*. whereby chlorophyll is virtually insensitive to low doses gamma irradiation. Abu *et al* stated that an increase in chlorophyll a, b and total chlorophyll levels was observed in *Paulownia tomentosa* plants that were exposed to gamma irradiation.

CONCLUSIONS

The results of the above research shown different effects on different parameters like germination frequency, seedling growth, proline, protein and chlorophyll contents. Protein, proline and chlorophyll contents were increased by irradiation. This technique can be used for the production of a mutant which has the ability for environmental stress tolerance.

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