Radiation Sensitivity of *Cajanus Cajan* to Gamma Radiations

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Abstract

Gamma irradiation induces various physiological, biochemical alterations in plants with modulation of certain metabolic and defensive pathway. Pre-sowing seed irradiation is considered as an effective method of improving production, yield components and chemical composition in plants. In the present study *Cajanus Cajan* was subjected to gamma irradiation with absorbed doses 0 Gy, 30 Gy, 50 Gy, 100 Gy, 150 Gy and 200 Gy with a dose rate 2.08 Kilo Gray per hour (2.08 K Gh⁻¹). *Cajanus cajan* when exposed to variable doses of gamma radiation showed persistent changes in the growth and development under both in vivo & in vitro conditions. Radiation sensitivity test based on germination percentage of irradiated and non-irradiated seeds demonstrated that significant reduction in germination percentage was observed with increasing gamma dosage under both in vivo and in vitro conditions. Biochemical analysis confirmed that protein, photosynthetic pigments, proline are very sensitive to gamma radiation, and are good indicators of tolerance. Effective stimulatory dose for plant development under in vivo conditions is 100 Gy while the absorbed doses of 150 Gy and 200 Gy can prove detrimental. However under in vitro conditions, results hold 150 Gy as threshold dose for increasing plant growth, plant vigour and development. Conclusively productivity of *Cajanus cajan* and consequent economic gains could be enhanced through adoption of suitable cultivar and level of gamma radiation. Gamma rays prove to be an important tool in increasing the breeding efficiency and regeneration frequency, especially that of the recalcitrant varieties. Results in the present study provide sufficient evidence to the effect that γ-irradiation does activate a biochemical system.

Keywords: Gamma rays; Radiation sensitivity; Proline; Total soluble protein; *Cajanus cajan*

Introduction

Unlike conventional breeding procedures which involve the production of new genetic combinations from already existing parental genes, nuclear technology causes exclusively new gene combinations with high mutation frequency. Mutation induction with radiation is most frequently used method to develop direct mutant varieties, as improvement with limited genetic variation. The first attempts to stimulate plant growth by exposing seeds or growing plants to optimum doses of ionizing radiation or by the use of radioactive fertilizers, dates back to the 1960s [1]. The use of the ionizing radiation technology may be considered as a revolution in agronomic research, especially in the plant protection, plant breeding and crop production [2,3]. Gamma-rays fall into the category of ionizing radiation and interact with atoms or molecules to produce free radicals in cells [4].

Gamma irradiation induces various physiological and biochemical alterations in plants. Gamma irradiation leads to changes in the plant cellular structure and metabolism [5,6]. Gamma-irradiation can be useful for the alteration of one or a few physiological characters [7]. Several positive mutations have been created in agricultural crops by using gamma irradiations. Crops with improved characteristics have successfully been developed by mutagenic inductions [8–10] like high yielding barley variety with early maturity, high protein contents and consequent economic gains could be enhanced through adoption of suitable cultivars [13–19] concluded that physical methods for processing of pre-sowing seed stimulates physiological and biochemical changes in the seeds. Studies by Deaf and Zheljazkov et al. [20–24] have also been carried out to elucidate the effects of gamma rays on some aromatic plants and legumes.

The aim of the present investigation was to assess the use of gamma radiation as a physical elicitor to alter the physiological characteristics of *Cajanus cajan* after exposures of pre-sowing seeds to variable doses of gamma rays under in vivo and in vitro conditions.

Materials and Methods

*Cajanus cajan* L. was selected for the present study. The healthy and authentic seeds were obtained from Division of Genetics (Pulse research) Indian Agricultural Research Institute, New Delhi. *Cajanus cajan* L. is a perennial member of the family Fabaceae. It ranks sixth in the area of production in comparison of other grain legumes & is one of the most valuable legumes grown in semi-arid and sub-tropical areas of the world. It is used in more diverse ways than others & exhibits notable pharmacological effects.

For *in vivo* and *in vitro* studies, seeds were irradiated with gamma radiation of absorbed doses 0 Gy, 30 Gy, 50 Gy, 100 Gy, 150 Gy and 200 Gy. The device used was Gamma Cell GC-5000 BRIT–BOMBAY. The source of gamma radiation was Cobalt-60; with a dose rate 2.08 Kilo Gray per hour (2.08 K Gh⁻¹) at Indian Institute of Nuclear Medicine and Allied Sciences (INMAS) New Delhi. Seed germination rate under both

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in vivo and in vitro conditions was carried out by radiation sensitivity test to determine the germination percentage after exposure to the gamma radiation. Germination percentage (GP) was calculated by using the following formula:

\[
GP = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sowed}} \times 100
\]

Fully opened leaves of individual plants were counted. The area of leaves was measured by using a leaf area meter (model 3000A, LICOR, USA) in centimeters (cm). Numbers of branches were counted at different stages of plant growth. The root and shoot length were measured in centimeters. For biomass of the root, leaves, and stem the samples were oven dried separately at 80°C for 48 hrs and dry weight was determined on a digital balance. Percent dry wt. of samples was calculated by using following formula:

\[
\% \text{ dry weight} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100
\]

For biochemical analysis, chlorophylls and carotenoids were measured from the fresh leaf by the method of Hiscox and Israelam [25]. The total soluble protein content of different sample explants was estimated following the method of Bradford [26]. The soluble protein concentrations were quantified with the help of standard curve prepared from the standard of bovine albumin serum (BSA) from sigma, USA. The protein content was expressed in mg g⁻¹ fr wt. The proline content was estimated by the method of Bates et al. [27]. The corresponding concentration of proline was determined against the standard curve processed in the same manner using L-proline (sigma). The amount of proline was expressed as μg g⁻¹ fr wt.

Results

The results for radiation sensitivity test based on germination percentage carried out under in vivo & in vitro conditions demonstrated significant reduction in germination percentage with increasing absorbed doses of gamma radiation. On an average, the mean germination percentage was greater for control, 30 Gy, 50 Gy and 100 Gy and lowest for 150 Gy and 200 Gy (Figure 1). For the germination percentage of irradiated seeds to reach 50%, the gamma dosage administered was 200 Gy. Under in vivo studies among the vegetative traits, root shoot ratio, shoot dry weight, total number of branches per plant showed a significant (p<0.05) increase with plant age in control as well as in treated plants (Tables 1-3). With increasing absorbed doses of gamma radiation, the vegetative traits showed linear increase up to 100Gy and thereafter showed a significant decrease with increase in absorbed doses.

A significant variation in growth was observed micro-shoots attained from gamma irradiated cultures were compared with respect to control (without irradiation) (Table 4). Under in vitro studies, the growth response of directly regenerated plantlets showed a significant (p<0.05) increase with plant age in controls as well as in treated plants (Figure 2). Increase in number of shoots up to 4 weeks showed a direct co-relation with increasing absorbed doses of gamma radiation as compared to control. However, after 4-weeks due to necrosis, plantlets at absorbed dose 200 Gy were not able to withstand irradiation effects, leading to decrease in the number of shoots. Maximum numbers of shoots were noted at the absorbed dose of 150 Gy, which produced about 9 shoots / explant at 12 weeks of culture while minimum number of shoots was noted at 30 Gy (Figure 4). In case of leaf number, maximum number of leaves / explant were noted at absorbed dose 150 Gy with 10 leaves / plant while minimum number of 5 leaves / plant at 30 Gy were observed (Table 1).

Number of seeds per plant and weight of 100 seeds showed significant (p<0.05) increase up to the absorbed dose of 100 Gy (Figure 3). Absorbed doses (30 Gy, 50 Gy & 100 Gy) reflected a stimulatory effect of gamma irradiation on number of seeds as compared to non-irradiated (control) (Figure 5). The maximum enhancement was observed with 50 Gy & 100 Gy. However, a significant decrease in number of seeds was observed with increasing doses of gamma radiation. Maximum decline was observed with 150 Gy & 200 Gy. Maximum variation of 39.09% was observed with 100 Gy followed by 23.05% at 50 Gy. Weight of 100 seeds exhibited the similar trend with maximum variation of 97.05% with 100 Gy and minimum variation of 22.06% with 200 Gy (Table 5).

Chlorophyll ‘a’ content enhanced significantly (p<0.05) with increasing doses of gamma radiation under both in vitro and in vivo conditions up to flowering stage and thereafter declined steadily (Figure 6). However Chlorophyll ‘b’ content declined significantly (p<0.05) with increasing doses of gamma radiation under both in vitro and in vivo conditions (Figure 6). A dosage dependent significant (p<0.05) increase in total chlorophyll content was also found under in vitro and in vivo conditions. A dosage dependent significant (p<0.05) increase in carotenoid content was also found under in vitro and in vivo conditions. A dosage dependent significant (p<0.05) increase in soluble protein content was found under in vitro and in vivo conditions (Figure 7). Proline content in both in vivo & in vitro conditions showed a significant (p<0.05) increase with increasing doses of gamma radiation (Figure 8).

Discussion

Radiation sensitivity test is a prerequisite step before the mutagenic treatment is started. The main purpose of this test is to investigate the most effective dosage of irradiation to be used and also to estimate the frequency and mutation spectrum using gamma irradiation. The results in the present study for radiation sensitivity test based on germination

Figure 1: Radiation sensitivity test to determine the germination percentage after exposure to the gamma irradiation
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Treated with different doses of Gamma radiations under *cajanus cajan* in *vivo* conditions

Table 1: Variation in shoot dry weight (gms) in *Cajanus cajan* at various growth stages treated with different doses of Gamma radiation under *in vivo* conditions

| Treatments | Developmental stages |
|------------|---------------------|
|            | Pre- Flowering      | Flowering | Post- Flowering |
| Control    | 0.24 ± 0.01         | 0.28 ± 0.01 | 0.33 ± 0.01 |
| 30 Gy      | 0.25 ± 0.01         | 0.31 ± 0.01 | 0.35 ± 0.01 |
| 50 Gy      | 0.27 ± 0.01         | 0.34 ± 0.01 | 0.37 ± 0.01 |
| 100 Gy     | 0.27 ± 0.01         | 0.35 ± 0.01 | 0.38 ± 0.01 |
| 150 Gy     | 0.24 ± 0.01         | 0.28 ± 0.01 | 0.31 ± 0.01 |
| 200 Gy     | 0.23 ± 0.01         | 0.27 ± 0.01 | 0.29 ± 0.01 |

"*P ≤ 0.05 CD at %* The values represent Mean ± SE (n=3)

Parenthesis shows percent variation.

Table 2: Variation in root: shoot ratio in *cajanus cajan* at various growth stages treated with different doses of Gamma radiation under *in vivo* conditions

| Treatments | Developmental stages |
|------------|---------------------|
|            | Pre- Flowering      | Flowering | Post- Flowering |
| Control    | 4.10 ± 0.11         | 4.62 ± 0.12 | 4.90 ± 0.13 |
| 30 Gy      | 5.04 ± 0.13         | 5.34 ± 0.14 | 5.74 ± 0.13 |
| 50 Gy      | 5.74 ± 0.15         | 5.98 ± 0.16 | 6.10 ± 0.16 |
| 100 Gy     | 5.86 ± 0.10         | 6.10 ± 0.11 | 6.38 ± 0.11 |
| 150 Gy     | 3.39 ± 0.09         | 3.95 ± 0.10 | 4.14 ± 0.11 |
| 200 Gy     | 3.08 ± 0.09         | 3.75 ± 0.09 | 4.00 ± 0.10 |

"*P ≤ 0.05 CD at %* The values represent Mean ± SE (n=3)

Parenthesis shows percent variation.

Table 3: Variation in total number of branches per plant in *Cajanus cajan* at various growth stages treated with different doses of Gamma radiation under *in vivo* conditions

| Treatments | Developmental stages |
|------------|---------------------|
|            | Pre- Flowering      | Flowering | Post- Flowering |
| Control    | 6.00 ± 0.16         | 8.50 ± 0.23 | 9.00 ± 0.24 |
| 30 Gy      | 7.50 ± 0.13         | 10.00 ± 0.27 | 11.50 ± 0.20 |
| 50 Gy      | 9.00 ± 0.24         | 12.50 ± 0.22 | 15.50 ± 0.42 |
| 100 Gy     | 9.05 ± 0.29         | 12.50 ± 0.16 | 16.00 ± 0.14 |
| 150 Gy     | 4.50 ± 0.12         | 5.00 ± 0.14 | 6.50 ± 0.11 |
| 200 Gy     | 4.01 ± 0.11         | 5.00 ± 0.13 | 6.01 ± 0.10 |

"*P ≤ 0.05 CD at %* The values represent Mean ± SE (n=3)

Parenthesis shows percent variation.

A decreasing trend in plant biomass with increasing gamma dose exposure suggests that there was radiation effect on carbon gain. Reduced carbon gain following higher gamma dosage has also been supported by various authors [34-36]. Decrease in the number of branches and leaf following higher gamma dosage from flowering to post-flowering stages is distinct. This might be due to premature abscission, induced as a result of increased production of ethylene [37-39]. Similar variability in seeds / pods has been recorded by Sharma et al. [40-42]. The increased average leaf area per plant would be expected to enhance the rate and efficiency of photosynthesis, which leads to a marked rise in plant biomass and consequently, would be associated with improved productivity. Authors Dubey et al. [41-45] have reported increment in seed yield following gamma radiations. At higher doses, number of seeds per plant decreased significantly. In the previous research, similar findings have also been reported by Sharma et al. in green gram, Charumathi, et al. in black gram and Guptas and Sharma in horse gram.

Chlorophyll content in the present study, showed a dosage dependent significant increase under *in vitro* and *in vivo* conditions which are in accordance with the results of Alilkananoglu et al. [46-47]. In addition, it was also observed that the concentration of chlorophyll *a* was relatively higher than chlorophyll *b* in irradiated and non-irradiated plants. Modulation in photosynthesis in irradiated plants might partly contribute to increased growth with similar findings also been reported by Sharma et al. in green gram, Charumathi, et al. in black gram and Guptas and Sharma in horse gram.

Following higher doses of gamma radiation, there was increase in total carotenoid content in both *in vivo* & *in vitro* conditions. Carotenoids function both as photosynthetic pigments and endogenous antioxidants, absorbing surplus energy and quenching active oxygen in addition to protecting chlorophyll by absorption of photon energy Casarett et al. [53-56].

In this study, it was found that there was an irregular distribution of total soluble protein content in irradiated plantlets under both *in vivo* and *in vitro* conditions. According to the results obtained in the present study, under *in vivo* conditions, it was observed that absorbed doses (150 Gy & 200 Gy) displayed lower total soluble protein content. However, under *in vitro* conditions soluble protein content showed a linear increase with increasing doses of gamma radiation. These results are in accordance with Cho and Song who observed that gamma irradiation did not induce significant loss in water soluble components such as total soluble proteins. Some investigators have observed slight depression or increase, while others reported no significant changes. During gamma irradiation of tomatoes, protein synthesis was not stopped but restored to form different set of proteins called as gamma induced proteins. The function of these is not yet known, but they may be involved in physiological disorders triggered by irradiation in repair process.
In the present study, proline content in both in vivo & in-vitro conditions showed an significant increase with increasing doses of gamma radiation which are in accordance with the findings of Esfandiari et al. [57]. Gamma irradiation leads to modulation of certain metabolic and defensive pathways. One of the protective mechanisms is the synthesis of osmolytes which is essential to plant growth in proline synthesis. Gamma irradiation at certain doses promotes the level of antioxidants. Gamma radiation at higher doses induce oxidative stress with overproduction of reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals and hydrogen peroxide, which react rapidly with almost all structural and functional organic molecules, including proteins, lipids and nucleic acids causing disturbance of cellular metabolism [58-60].

**Conclusion**

Conclusively productivity of *Cajanus cajan* and consequent economic gains could be enhanced through adoption of suitable cultivar and level of gamma radiation. Results in the present study provide sufficient evidence to the effect that γ-irradiation does activate a biochemical system. Biochemical analysis confirmed that the differences between various cultures in their ability to accumulate such compounds were evident under stress but not under controlled conditions. Yield parameters showed a significant enhancement with
Figure 3: Variation in of formation of pods under in vivo conditions after treatment with different doses of gamma radiation. A: control; B: 30Gy; C: 50Gy; D: 100Gy; E: 150Gy; F: 200Gy

Figure 4: Increase in callus formation / regeneration frequency after irradiation with different doses of gamma radiation. A: Callus growth in controlled cultures; B: Callus growth in irradiated cultures; C: Induction of shoots in controlled cultures; D: Induction of shoots in irradiated cultures; E: Induction of multiple shoots in controlled cultures; F: Induction of multiple shoots in irradiated cultures.

Figure 5: Variation in in vitro yield attributes in Cajanus cajan at different doses of gamma radiation absorbed dose of 100 Gy. Optimum doses of ionizing radiations have modulatory role in the growth and developmental processes. Effective stimulatory dose for plant development under in vivo conditions is 100 Gy while the absorbed doses of 150 Gy and 200 Gy can prove detrimental. However under in vitro conditions, results hold 150 Gy as threshold dose for increasing plant growth, plant vigour and development. Gamma rays prove to be an important tool in increasing the breeding efficiency, and regeneration frequency, especially that of the recalcitrant varieties. In this context, further work is required where the response of plant to different doses of gamma radiation can
be elaborated.

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