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GAMMA IRRADIATION INFLUENCE ON SEED GERMINATION, EARLY SEEDLING GROWTH AND PHENOTYPIC VARIATIONS IN *BAUHINIA PURPUREA*

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ABSTRACT

Air dried seeds were exposed to continuous and fractionated doses of gamma radiation at dosage levels of 10KR, 20KR, 40KR, and 80KR in an experiment to measure germination and early seedling growth pattern change in *Bauhinia purpurea*. Seeds were pre-soaked for 24 hours at room temperature (27.5%-1.700°C) after treatment. Seeds were scarred for proper imbibition prior to pre-soaking. A petri plate with Whatman filter paper no. 1 and different dosages of seeds was used. Lab temperatures ranged from 240°C to 340°C. There were 50 seeds in each set, each with four replications. Results from the irradiation and control treatments showed a discernible difference.

With the exception of the cotyledons growth dimension, almost all dose level treatments decreased the measured parameters when compared to untreated sets (control). Following continuous and fractionated treatments at dose levels of 10, 20, and 80 KR, the Control set showed the highest levels of GP, GV, and GEI. In comparison to lower dose levels (10&20KR), the higher doses (40 & 80KR) were more effective at lowering the germination value.

For various doses, *Bauhinia purpurea's* hypocotyl was more radio resistant than its roots. The leaves and the shoot displayed greater radio sensitivity. Under an 80KR dosage level, there were no shoots or leaves. Gamma radiation fractioned dosages were more successful at preventing *Bauhinia purpurea* seedling germination and growth. LD-50 readings varied depending on the parameters that were examined.

Keywords : *Bauhinia purpurea,* gamma irradiation, Continuous and fractionated doses, LD_{50}

Introduction

Gamma radiation is one of the physical mutagen employed in mutant breeding in plant species to cause diversity and in tree species (De Micco *et al.,* 2011 Moussa, 2011; Iglesias *et al.,* 2012). According to Ikram *et al*. (2010), depending on the intensity of irradiation, it induces genetic, cytological, biochemical, physiological, and morphogenetic alterations in plant cell and tissues. Gamma rays from ionising radiation interact with atoms and molecules inside of cells to produce free radicals. These radicals may result in genetic mutation since they can seriously harm DNA or chromosomes inside of cells.

Knowing the dose rate of gamma rays and the condition of treatment has become crucial. As is well known, different tree species respond differently to gamma rays. To illustrate this, the fatal dosage that causes a 50% reduction (LD_{50}) has been utilised extremely well and is thought to be an appropriate dose for initiating mutation in the case of a physical mutagen. Tree seeds' dormancy is broken by gamma radiation treatment, which also promotes early germination and seedling growth.

The *Bauhinia purpurea* is a member of the Fabaceae family. It is native to Myanmar and the Indian subcontinent and has been widely spread to other tropical and subtropical regions of the world. It is only epigeal in origin. The young shoot develops between the cotyledons and the radicle already emerges out as the plumule starts to grow. Later, the young shoot separates very slightly and extricates itself by bending or arching as in hypogeal germination, but after its emergence, the cotyledons, which turn green, separate and are carried above ground on short hypocotyl.

Materials and Methods

The *Bauhinia purpurea* seeds were gathered in the Tehri Garhwal near Dhanolti. The seeds were removed from the pods, air dried, and kept in glass vials between 24 and 36 degrees Celsius. Acute gamma radiation was used to irradiate the air-dried seeds at dose rates of 100 rad/sec 60Co at dose levels of 10, 20, 40, and 80KR. The doses were administered in two different ways. The desired dose was administered continuously to administer the continuous doses. The divided doses were administered in two equal portions with a 48-hour gap between each treatment. The seeds were sterilised in 10% sodium hypochlorite for 10 minutes, sterilised in tab water for 15 minutes, and then rinsed with sterile distilled water. The seeds were then slashed with a razor-sharp blade to aid with ingestion. As a control, unirradiated seeds were used. These seeds were later presoaked in distilled water for 24 hours together with control sets, at room temperature (27.51°C). The seeds were properly cleansed with distilled water after being presoaked. In petri plates using Whatman's

filter paper no. 1 and a temperature range of 24°C to 34°C, various dosages of seeds were maintained. There were 50 seeds in total, for each four replicates.

We noticed data for germination value, germination percentage, and germination energy index. By examining the radicle and hypocotyl, early seedling growth has been measured. By measuring the length, width, and area of the shoot and the expansion of the leaves at various doses, these changes have been documented.

The seedling's variations have been observed to have phenotypic variances.

The following germination information has been noted:

Germination Percent

Germination Value

Following is the formula for determining the Germination value (GV):

$$GV = MDG \times PV$$

(MDG = Mean Daily Germination)

PV = Peak Value

$$PV = \frac{\text{Germination Percentage}}{\text{Number of days}}$$

Germination Energy Index

The germination data for calculating the Germination Energy Index was recorded up to when it became constant for three consecutive days. Up to this day, the figures were used as the reference point and the germination obtained for all the treatments were used for computing the GEI, using the modified formula:

$$GEI = \frac{A1 + (A1 + A2) + (A1 + A2 + A3) + (A1 + A2 + A3 + ...An)}{n \times N} \times 100$$

where, A_1. A_2 A_3 -- -A_n represents the number of seeds newly germinated on 1st, 2nd, 3rd and nth day, respectively. N is the total number of seeds used for experiment and n is the number of days under observation.

Seedling Growth

Data of radicle, hypocotyl, shoot growth were recorded. The phenotypic variations and mutant types have also been observed.

Results

Different continuous and fractionated gamma ray dosages have an effect on the germination percentages of *B. purpurea*. Compared to treated seeds, untreated seeds exhibited a higher rate of germination. The GP falls as the gamma dose rate rises from 10 KR to 80 KR for continuous and fractionated treatment. Maximum GP (64.62%) and minimum GP (45.50) have been observed for control sets (seeds not treated). When compared to lower doses, the germination percentage significantly decreased with higher gamma radiation exposure. (Table 1&2)

Tables 3 and 4 provide evidence that the germination value for *B. purpurea* is more gamma-ray sensitive than the germination percentage and germination energy index (Tables 5 and 6). Every gamma radiation dose evaluated lowers the Germination Value. The Germination Value decreases in tandem with an increase in dose rate. Maximum germination value reduction (72.47%) was seen when 80KRF was used as a treatment. For continuous and fractionated doses, the LD-50 for Germination Value has been measured as 67KR and 39KR, respectively. At a 5% level, the statistical values were very significant. It's also noteworthy to observe that *B. purpurea* seedlings exhibit the similar pattern of decrease over untreated seeds in the Germination Energy Index. The GEI value drops as the dose rate of gamma rays for continuous and fractionated radiation rises. The LD-50 for the germination energy index in *B. purpurea* has not been determined, although the lowest (41.51) GEI has been recorded under 80KR continuous dose level, followed by 80KR fractionated dose (41.68). After receiving gamma radiation therapy. Seedlings radicle lengths displayed a nearly linear trend for reduction values (Tables 7 and 8). The fractionated doses, however, once produced more inhibition than the continuous doses. All tested dosages reduce radicle length as the dose rate rises. The colour of the radicle likewise changes and turns brownish-black. When exposed to 80KRC and 80KRF, *B. purpurea's* root tip suffered full destruction and developed a brown tint.

Table 1 : *Bauhinia purpurea*–Germination Percentage of seedlings as influenced by different continuous doses of gamma rays.

S.No.	Treatments	GP
1	Control	64.62
2	10 KR	58.54
3	20 KR	53.92
4	40 KR	51.50
5	80 KR	45.50

Table 2 : *Bauhinia purpurea*–Germination Percentage of seedlings as influenced by different fractionated doses of gamma rays

S.No.	Treatments	GP
1	Control	64.62
2	10 KR	55.67
3	20 KR	51.75
4	40 KR	52.00
5	80 KR	45.50

Table 3: *Bauhinia purpurea*–Germination Value of seedlings as influenced by different continuous doses of gamma rays.

S.No.	Treatments	GV
1	Control	258.95
2	10 KR	231.60
3	20 KR	147.93
4	40 KR	146.39
5	80 KR	123.41

Table 4: *Bauhinia purpurea*–Germination Value of seedlings as influenced by different fractionated doses of gamma rays.

S.No.	Treatments	GV
1	Control	258.95
2	10 KR	133.36
3	20 KR	174.42
4	40 KR	128.71
5	80 KR	86.31

Table 5: *Bauhinia purpurea* – Germination Energy Index of seedlings as influenced by different continuous doses of gamma rays.

S.No.	Treatments	GEI
1	Control	61.65
2	10 KR	54.62
3	20 KR	45.11
4	40 KR	49.98
5	80 KR	41.51

Table 6: *Bauhinia purpurea* – Germination Energy Index of seedlings as influenced by different fractionated doses of gamma rays.

S.No.	Treatments	GEI
1	Control	61.65
2	10 KR	50.59
3	20 KR	63.87
4	40 KR	43.33
5	80 KR	41.68

Table 7: *Bauhinia purpurea* – radicle length (cm) of seedlings as influenced by different continuous doses of gamma rays.

S.No.	Treatments	Radicle Length (cm)
1	Control	2.41
2	10 KR	2.35
3	20 KR	2.25
4	40 KR	2.04
5	80 KR	1.31

Table 8: *Bauhinia purpurea* radicle length (cm) of seedlings as influence by different fractionated doses of gamma rays.

S.No.	Treatments	Radicle Length (cm)
1	Control	2.41
2	10 KR	2.25
3	20 KR	1.71
4	40 KR	1.39
5	80 KR	1.37

Table 9: *Bauhinia purpurea* – hypocotyl length (cm) of seedlings as influenced by different continuous doses of gamma rays.

S.No.	Treatments	Hypocotyl Length (cm)
1	Control	0.52
2	10 KR	0.90
3	20 KR	0.52
4	40 KR	0.49
5	80 KR	0.44

Table 10: *Bauhinia purpurea* – hypocotyl length (cm) of seedlings as influenced by different fractionated doses of gamma rays.

S.No.	Treatments	Hypocotyl Length (cm)
1	Control	0.52
2	10 KR	0.70
3	20 KR	0.49
4	40 KR	0.44
5	80 KR	0.41

Table 11: *Bauhinia purpurea* – shoot length (cm) of seedlings as influenced by different continuous doses of gamma rays.

S.No.	Treatments	Shoot Length (cm)
1	Control	7.81
2	10 KR	7.18
3	20 KR	5.76
4	40 KR	2.55
5	80 KR	0.72

Table 12: *Bauhinia purpurea* – shoot length (cm) of seedlings as influenced by different fractionated doses of gamma rays.

S.No.	Treatments	Shoot Length (cm)
1	Control	7.81
2	10 KR	6.53
3	20 KR	2.73
4	40 KR	2.43
5	80 KR	2.06

Table 13: *Bauhinia purpurea* – area of leaf (mm) of seedlings as influenced by different continuous doses of gamma rays.

S.No.	Treatments	Leaf area (mm²)
1	Control	319.75
2	10 KR	257.25
3	20 KR	197.75
4	40 KR	177.25
5	80 KR	**

** - No leaf formation

Table 14: *Bauhina purpurea*- area of leaf (mm) of seedlings as influenced by different fractionated doses of gamma rays.

S.No.	Treatments	Leaf area (mm²)
1	Control	319.75
2	10 KR	203.2
3	20 KR	168.25
4	40 KR	150.75
5	80 KR	**

** - No leaf formation

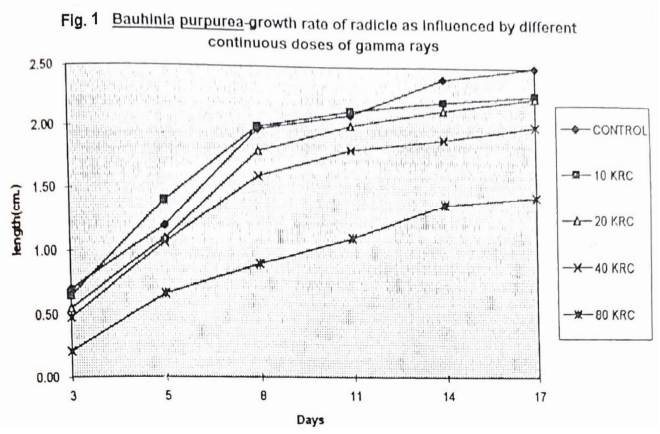


Fig. 2 Bauhinia purpurea - growth rate of radicle as influenced by different fractionated doses of gamma rays.

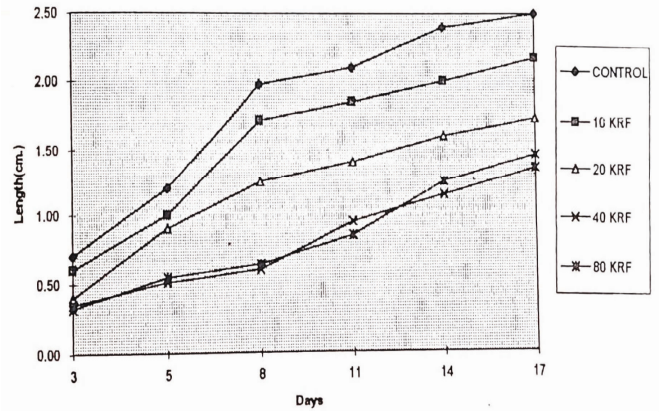


Fig. 3 Bauhinia purpurea - growth rate of hypocotyl as influenced by different continuous doses of gamma rays.

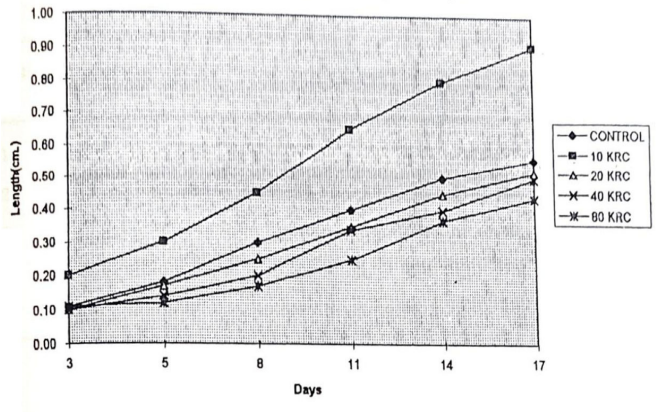


Fig. 4 Bauhinia purpurea - growth rate of hypocotyl as influenced by different fractionated doses of gamma rays.

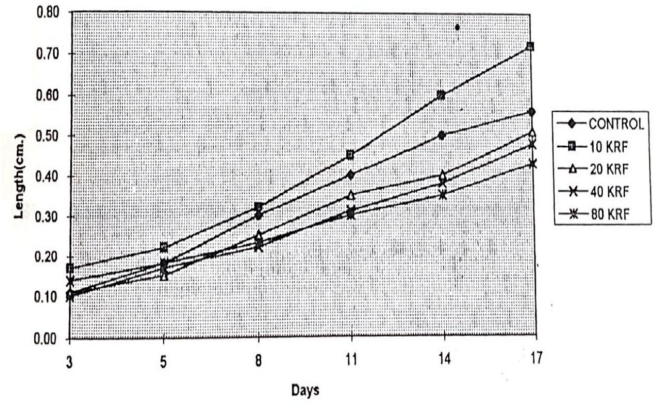


Fig.5 . Bauhinia purpurea - growth rate of shoot as influenced by different continuous doses of gamma rays

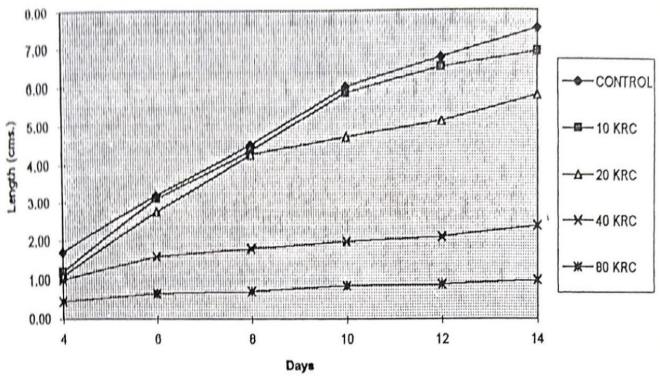
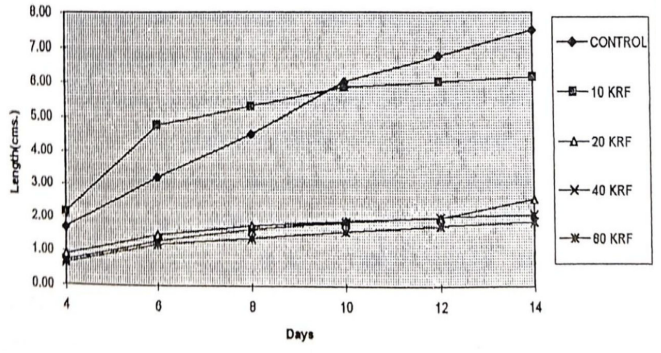


Fig.6 . Bauhinia purpurea - growth rate of shoot as influenced by different fractionated doses of gamma rays



B. purpurea seedlings' hypocotyl length was discovered to be less vulnerable to gamma radiation than their radicle length. Only the smaller doses (18KRC and 10KRF) promoted the length of the hypocotyl. The 10KRC therapy produced the maximum stimulation (80.76%) for hypocotyl length, followed by the 10KRF treatment (34.61%). It was discovered that the hypocotyl of *B. purpurea* was more susceptible to fractionated doses of gamma radiation than continuous doses. Under the therapy of an 80KR fractionated dose, the highest reduction percentage (21.15%) for hypocotyl development was noted (Tables 9&10).

Gamma ray treatment has a significant impact on *B. purpurea's* shoot shape and growth behaviour. The shoot length of the seedlings receiving various dosages of treatment exhibits a linear trend of shortening. It was discovered that fractionated doses worked better to stop the growth of the shoot. The shoot and leaf production in the *B. purpurea* seedlings has been entirely suppressed by the 80KRC and 80KRF dosages. For continuous and fractionated dosages, the LD_{50} for the shot length was reported as 30KR and 16KR, respectively, and the differences were statistically significant (Tables 11&12).

At an early stage of seed germination, the growth rate of the radical, hypocotyl, and shoot has been evaluated for its radio sensitivity. The growth rate was highest under control sets (Fig. 1 to Fig. 6) but the 10 and 20 KR dosages of continuous and fractionated treatments indicate superior growth rate among all treatments of gamma rays for radicle, hypocotyl, and shoot.

Different continuous and fractionated gamma ray dosages have had a significant impact on the area of the foliage leaves. In terms of continuous dosages, it was discovered that the 40KR dose decreased the area by up to 44.56% in comparison to control. The leaf area was shown to be more effectively reduced by fractionated doses, and under the 40KR treatment, a 52.85% reduction was noted. Under the treatment of 80KR continuous and fractionated doses, there were no leaf or shoot formations (Table 13 &14).

Discussion

By measuring reduction values above controls and taking measurements of several growth parameters, the impact of gamma rays was observed. The outcome demonstrates that, in compared to the control set, germination percent, germination value, and germination energy index were decreased for all dosages under

continuous and fractionated treatment. Gamma rays have been proven to reduce germination in *Albizia lebbek* and stimulation at lower dose level for cotyledonary leaves dimension and shoot growth (Singh & Paliwal, 1987). *Pinus roxburghii, P. patula, Eucalyptus alba*, and *E. grandis* were studied by Kapoor (1981), who observed similar results about the diminished effects of gamma rays on GP, GV, and GEI. *Plantago ovale*, Turkish sesame, *Braasica nepas*, and sugar cane were among the crop plants for which reduced germination data were also seen (Sareen & Kaul, 1999; Cagirgan, 1996; Zhou Yongming *et al*., 1998; Singh *et al*., 1993).

As a result of gamma radiation, meristematic cells are injured and their metabolic activity is disrupted, which leads to decreased germination and growth (Gunckel, 1965; Maherchandani, 1975; Grover & Dhanju, 1979). With the exception of hypocotyl growth at 10KRF level, all continuous and fractionated gamma ray doses in *B. purpurea* reduced radical, hypocotyl, and shoot growth. As a result, the growth of the radicle and shoot following treatment may be delayed. Protein production in the embryonic cell may also inhibit passage of the cell from G1 forward. Contrarily, stimulatory tendencies were noted in *Dalbergia sissoo* (Bhandari, 1984), and *Acacia catechu* (Rawat and Sharma, 1996).

Low amounts of gamma radiation may promote the young embryo's enzyme activation and waking, which stimulates cell division and influences both germination and vegetative growth (Sjodin, 1962). In contrast, several studies have shown that gamma rays have inhibitory effects at higher dose levels and that, for the most part, low doses of radiation encourage germination and growth in a variety of tree species (Singh & Sujata, 2004; Singh & Vandana, 2008).

According to Wi *et al*. (2007), Ashraf (2009), the primary cause of the biological effects of gamma irradiation is the production of free radicals from the hydrolysis of water, which may modulate an anti-oxidation system and cause a buildup of phenolic compounds and chlorophyll pigments.

The genetic loss caused by chromosomal abnormalities and gene mutations can be blamed for stunted growth and decreased survival (Sparrow *et al*., 1968). Plant growth hormone degradation may also be a contributing factor in the reduction of early seedling growth (Haque & Godward, 1984). According to Agrawal and Kaul (1998), irradiation treatment inhibits the production of normal cellular protein and induces a new set of protein synthesis to begin, which is what causes phenotypic differences.

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