Original Research Article

Gamma irradiation - A tool for enhancing storage life of grain cowpea (*Vigna unguiculata* (L.) Walp.)

Abstract

An experiment was conducted at the Department of Seed Science and Technology, College of Agriculture, Vellayani to study the influence of gamma irradiation in enhancing storage life of grain cowpea. The experiment consists of six treatments (100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy and control) with different doses of gamma rays. All the treatments were effective in control of pulse beetle infestation without any seed damage and consequently no weight loss compared to control with 56.333, 28.182 per cent seed damage and weight loss. The treatments showed higher germination parameters compared to control at lower doses of gamma rays. The treatment T_2 (200 Gy) recorded highest seed germination percentage (84.33%). Speed of germination, seedling shoot length and seedling dry weight was highest at T_2 (200Gy) with 32.13, 11.83 cm and 0.703 g. Seedling root length was highest at T_1 (100 Gy) with 13.84 cm. Seedling vigour index I and II were maximum at T_2 (200 Gy) with 2130.49 and 59.28. In the field experiment, progressive decrease in all morphological parameters was observed with increase in doses of beyond 200 Gy. No significant variation was observed in 100 Gy, 200 Gy and control. However, among all the gamma doses, 200 Gy (T_2) recorded the least seed damage, weight loss and improvement in germination compared to control.

Keywords: Cowpea seeds, Gamma irradiation, Seed storage, Seed germination, Pulse beetle

1. Introduction

Seed is the fertilized, matured ovule and a carrier of genetic potential for sustainable crop production. It is the basic and crucial input of agriculture around which all other input acts. Cowpea [*Vigna unguiculata* (L.) Walp.] also called black-eyed pea, lobia, barbati is a widely grown leguminous crop (2n=22). The requirement of cowpea has been increasing over decades but there was a deficit in cowpea production in comparison with the demand by the growing population. In addition to the shortage of production, the stored product was also affected by the number of various biotic and abiotic factors. Among them, insect pests are of economic concern as they contribute to nearly 10- 50 per cent of damage and damage loss. Apart from them, mites, rodents, birds, and microbes also cause great loss. Stored pests that feed on seeds are the major biotic factor that causes considerable economic losses during storage. Pulse beetle (Bruchids), *Callosobruchus chinensis* is the most important pest as they multiply rapidly and cause heavy loss both in field and storage (Ahmed *et al.*, 2003)[1]. Synthetic insecticides and fumigants are often used in storage to combat pests, but their widespread use in the field and storage has resulted in a slew of issues, including insecticide resistance, poisonous contaminants in food crops, waste and rising application prices (Kumar *et al.*, 2013)[2]. Gamma irradiation of legumes has emerged as a new technology to combat the problems caused by the storage pests and helps to maintain its longevity in storage. Irradiation by use of gamma source Co 60 or Cs 135, X rays or high energy electrons provides an alternative to chemical treatment that leads to inherent problems like residues and environmental pollution (Farkas, 1998)[3]. Irradiation can potentially eliminate insect pests of stored grains as well as field crops. It is an eco-friendly technology for insect pest management, without causing any induced residual effect and radioactivity.

2. Materials and methods

The experiment was conducted in Department of Seed Science and Technology, College of Agriculture, Vellayani, Thiruvananthapuram to study the effects of gamma rays in enhancing storage life of grain cowpea. The experiment consists of six treatments viz T_1: 100 Gy, T_2: 200 Gy, T_3: 300 Gy, T_4: 400 Gy, T_5: 500 Gy, T_6: Control. The experiment was conducted in Completely Randomized Block Design (CRD) in three replications. For imposition of gamma rays, 600 grams of seed is used in each treatment. Irradiation was carried out at Indian Institute of Horticultural Research (IIHR), Bangalore. The irradiated seeds were packed in polythene bags and stored for six months at ambient room temperature. The monthly observations on various parameters were carried out and statistically analysed. The percentage seed damage (Eq. 3) was determined by collecting a sample of 100 seeds from each three replications of each treatment at monthly intervals

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Comment [D4]: how many degree? Please mention proposed by Adams and Schulten (1978)[4]. Seed weight loss percentage (Eq. 3) was calculated from number of damaged and undamaged seeds, weight of damaged and undamaged seeds as given by Adams and Schulten (1978). Number of eggs per 100 seeds was also calculated at monthly intervals. The germination test was carried out with 100 seeds in four replications with rolled paper towel method for 8 days (ISTA, 2013)[5]. Normal healthy seeds were taken for germination test. Other germination parameters like speed of germination (Maguire, 1962)[6] (Eq. 3), seedling shoot and root length, seedling dry weight, Seedling vigour index I (Eq. 4) and II (Eq. 3) (Abdul- Baki and Anderson, 1973) was calculated at monthly intervals. Irradiated seeds were randomly selected in each treatment and sown in field to evaluate any morphological abnormalities arising due to mutation effect. Seeds were sown with the spacing of 30×15 cm. The experiment was carried out in Randomized Block Design (RBD) with three replications. Various morphological parameters like germination percentage, plant height, number of pods per plant, number of seeds per pod, 100 seed test weight and morphological abnormalities were observed.

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$$\text{Eq(1)Percentage seed damage} = \frac{\text{Number of damaged seeds}}{\text{Total number of seeds taken}} \times 100$$

$$\text{Eq. (2)} \quad \text{Seed weight loss percentage} = \frac{U\ (Nd) - D\ (Nu)}{U\ (Nu+Nd)} \times 100$$

Nd- Number of damaged seeds
Nu- Number of undamaged seeds
D- Weight of damaged seeds
U- Weight of undamaged seeds

Eq. (3) **Speed of germination** = $X_1 / Y_1 + X_2 / Y_2 + \ldots\ldots\ldots\ldots\ldots + X_n - X_{n-1} / Y_n$

$X_{n\,=}$ percent germination on n^{th} day
$Y_{n\,=}$ number of days from sowing to n^{th} count

Eq. (4) **Seedling Vigor index I** = Germination (%) × Seedling length (cm)

Eq. (5) **Seedling Vigor index II** = Germination (%) × Seedling dry weight (g)

3. Results and discussion

Among the different treatments, all the gamma doses used were significantly superior to control in reducing infestation of cowpea seeds during storage. Gamma irradiation proves to be an alternative for pulse beetle control and these results were confirmed with the investigations carried out by Dongre *et al.* (1997)[7], Ahmad *et al.* (2003)[8] and Tripati *et al.* (2015)[9]. Seed damage was not observed in treatments T_1 (100 Gy) for up to three months of storage and T_2 (200 Gy) for up to five months of storage. No seed damage and insect eggs were observed in treatment T_3 (300 Gy), T_4 (400 Gy) and T_5 (500Gy) throughout the storage period. In contrast, seed infestation was observed from first month up to six months in control. At the end of storage period, percentage seed damage was highest in Control (56.33%) which was then followed by 100 Gy (2.667%) and 200 Gy (0.667%). In the same line, Darfour *et al.* (2012)[10] concluded that gamma rays of 250 Gy lead to 100 per cent mortality of *C. maculatus* within 8 days of irradiation. Exposure of green gram seeds infested with pulse beetle with different gamma doses ranging from 100 Gy to 500 Gy showed varying effects and at 100 Gy all the adult beetles were sterile (Bhalla *et al.*, 2008)[11]. Seed weight loss percentage was highest in control (28.182 %) which was then followed by 100 Gy (0.995 %) and 200 Gy (0.290 %). The results of the present study are in conformity with the findings of Enu and Enu (2014)[12] who reported that 300 Gy and 500 Gy gamma doses showed 100 per cent mortality of *Sitophilus zeamais* and *Callosobruchus maculatus.* The number of eggs laid progressively increased in control over the period of study. At the end of storage period, highest number of eggs was laid in control with the number of eggs ranging from 0.333 nos from the first month to 78.333 nos in the sixth month of storage

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Table 1: Effect of gamma rays on percentage seed damage, seed weight loss percentage, and number of eggs per 100 seeds

Treatment	Percentage seed damage (%)		Seed weight loss percentage (%)		Number of eggs per 100 seeds	
	3 MAS	6 MAS	3 MAS	6 MAS	3 MAS	6 MAS
T_1 (100 Gy)	0.000 (1.00)	2.667^b (1.816)	0.000 (1.00)	0.995^b (1.373)	0.000 (1.00)	4.333^b (2.150)
T_2 (200 Gy)	0.000 (1.00)	0.667^c (1.244)	0.000 (1.00)	0.290^b (1.122)	0.333 (1.138)	1.667^{bc} (1.577)
T_3 (300 Gy)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_4 (400 Gy)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_5 (500 Gy)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)	0.000 (1.00)
T_6 (Control)	2.000 (1.656)	56.333^a (7.558)	0.748 (1.303)	28.182^a (5.395)	3.000 (1.882)	78.333^a (8.903)
SE (m)	0.146	0.242	0.065	0.133	0.203	0.282
CD (5%)	NS	0.754	NS	0.414	NS	0.878

NS- Non significant **MAS- Months after storage**
Values in parenthesis are square root transferred values
The values in the same column with the same alphabet are not significantly different

Germination percentage was highly influenced by gamma rays. Irrespective of the treatments, germination percentage declined with increase in storage period. Susmitha and Rai (2017)[13] reported that a decrease in germination potential was due to the aging process that consequently leads to depletion of food reserves and seed deterioration. At the end of six months storage, T_2 (200 Gy) recorded highest germination per cent (84.33%), which was on par with T_1 (100 Gy) and T_6 (Control) with germination per cent of 81.80 per cent and 80.66 per cent. Also, T_5 (500 Gy) recorded the lowest germination per cent (26.33%) among the treated seeds as well as control seeds. The stimulatory effect of germination at lower doses of gamma rays was due to RNA activation and protein synthesis. A lower dose of gamma rays had a stimulatory effect on germination due to better oxygen uptake and dehydrogenase enzyme activity that provides energy to the embryo and thereby increases the overall metabolic activity. Speed of germination was highly influenced by gamma rays. Treatment T_2 (200 Gy) recorded the highest speed of germination (32.13) which was on par with T_1 (100 Gy) and T_6 (Control). Treatment T_5 (500 Gy) recorded the lowest speed of germination (26.33). Early reports of Akshatha and Chandrasekar (2013)[14] also support these findings. The lower dose of gamma rays (25 Gy) imposed a significant increase in the speed of germination in *Pterocarpus sp*. Treatment T_2 (200 Gy) recorded the highest seedling shoot length (11.83 cm), which was on par with T_6 (Control) with 11.56 cm and T_1 (100 Gy) with 11.50 cm. The least seedling shoot length (8.56 cm) was recorded in T_5 (500 Gy). The highest seedling root length (13.84 cm) was observed in T_1 (100 Gy) which was on par with T_2 (200 Gy) with 13.43 cm and T_6 (Control) with 12.45 cm. The least seedling root length (7.23 cm) was recorded in T_5 (500 Gy). Similar findings were reported by Pranesh *et al.* (2019)[15] in which a reduction in shoot length (5.1 and 5.9 cm) was observed at higher doses (1000 Gy) of gamma rays in Bengal gram and black gram at nine months of storage. The impact of gamma rays on germination parameters is given in Table 2.

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Table 2: Effect of gamma doses on germination percentage, speed of germination, seedling shoot length, and seedling root length

Treatment	Germination percentage (%)		Speed of germination		Seedling shoot length (cm)		Seedling root length (cm)	
	3 MAS	6 MAS	3 MAS	6 MAS	3 MAS	6 MAS	3 MAS	6 MAS

T_1 (100 Gy)	89.00^{a} (9.48)	81.80^{ab} (9.09)	35.33^{a} (6.03)	32.10^{a} (5.75)	13.83^{a} (3.85)	11.50^{ab} (3.53)	15.63^{a} (4.07)	13.84^{a} (3.85)
T_2 (200 Gy)	90.40^{a} (9.56)	84.33^{a} (9.23)	35.20^{a} (6.02)	32.13^{a} (5.76)	13.63^{a} (3.82)	11.83^{a} (3.58)	15.50^{a} (4.06)	13.43^{ab} (3.78)
T_3 (300 Gy)	86.30^{ab} (9.34)	78.19^{bc} (8.89)	31.10^{b} (5.67)	28.40^{b} (5.420	11.56^{bc} (3.54)	10.30^{bc} (3.36)	10.87^{b} (3.44)	9.17^{c} (3.18)
T_4 (400 Gy)	83.00^{bc} (9.16)	76.79^{cd} (8.81)	29.63^{c} (5.53)	27.60^{b} (5.35)	10.63^{cd} (3.40)	9.53^{cd} (3.24)	9.50^{c} (3.24)	8.46^{c} (3.07)
T_5 (500 Gy)	79.39^{c} (8.96)	72.60^{d} (8.57)	28.03^{c} (5.39)	26.33^{c} (5.23)	10.03^{d} (3.32)	8.56^{d} (3.09)	8.20^{d} (3.03)	7.23^{d} (2.86)
T_6 (Control)	88.19^{a} (9.44)	80.66^{abc} (9.03)	34.20^{a} (5.93)	31.40^{a} (5.69)	12.60^{ab} (3.67)	11.56^{ab} (3.54)	15.07^{a} (4.00)	12.45^{b} (3.66)
SE (m)	0.07	0.08	0.03	0.03	0.06	0.06	0.04	0.05
CD (5%)	0.227	0.269	0.095	0.114	0.192	0.187	0.151	0.162

MAS- Months after storage
Values in parenthesis are square root transferred values
The values in the same column with the same alphabet are not significantly different

Maximum seedling dry weight (0.703 g) was recorded in T_2 (200 Gy) which was on par with T_1 (100 Gy) with 0.693g, T_6 (Control) with 0.687 g and T_3 (300 Gy) with 0.641 g. The lowest value (0.549 g) was recorded by T_5 (500 Gy). The results are contradictory with Borzouei *et al.* (2010)[16] who reported that gamma doses of 100 Gy resulted in a 25 per cent increase in dry weight whereas 200, 300, and 400 Gy resulted in a decrease in dry weight compared to control. The reduced fresh and dry weight of shoot might be attributed to reduced moisture content or plant growth due to radiation stress (Majeed *et al.*, 2010). Maximum seedling vigour index I (2130.49) was observed in T_2 (200 Gy) which was on par with T_1 (100 Gy) with 2074.57 and T_6 (Control) with 1939.24. The lowest value (1146.47) was exhibited by T_5 (500 Gy). Highest seedling vigour index II (59.28) was recorded in T_2 (200 Gy) which was on par with T_1 (100 Gy) with 56.73 and T_6 (control) with 55.52. The lowest value (39.86) was observed in T_5 (500 Gy). This result was supported by Chandrashekar (2015) who found that seedling vigour index showed a two-fold increase at 50 Gy compared to control in *Canarium strictum.* Improvement in growth parameters may be due to enhanced photosynthesis that leads to an increase in carbohydrate content. The impact of gamma rays on seedling dry weight, seedling vigour index is given.

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Table 3: Effect of gamma doses on seedling dry weight, seedling vigour index I and seedling vigour index II

Treatment	Seedling dry weight (g)		Seedling vigour index I		Seedling vigour index II	
	3 MAS	6 MAS	3 MAS	6 MAS	3 MAS	6 MAS
T1	0.744^{ab} (1.320)	0.693^{a} (1.301)	2622.31^{a} (51.20)	2074.57^{a} (45.53)	66.14^{ab} (8.19)	56.73^{ab} (7.59)
T2	0.759^{a} (1.326)	0.703^{a} (1.305)	2635.00^{a} (51.33)	2130.49^{a} (46.17)	68.56^{a} (8.34)	59.28^{a} (7.76)
T3	0.721^{bcd} (1.312)	0.641^{ab} (1.281)	1935.13^{b} (44.00)	1522.68^{b} (39.03)	62.24^{c} (7.95)	50.14^{bc} (7.15)
T4	0.700^{cd} (1.304)	0.597^{bc} (1.264)	1672.02^{c} (40.89)	1383.03^{b} (37.19)	58.07^{d} (7.69)	45.89^{cd} (6.84)
T5	0.693^{d} (1.301)	0.549^{c} (1.245)	1447.06^{d} (38.05)	1146.47^{c} (33.87)	55.04^{e} (7.49)	39.86^{d} (6.39)
T6	0.728^{abc} (1.315)	0.687^{a} (1.301)	2438.30^{a} (49.37)	1939.24^{a} (44.02)	64.25^{bc} (8.07)	55.52^{ab} (7.51)
SE (m)	0.004	0.008	0.63	0.72	0.064	0.165
CD (5%)	0.0130	0.0260	1.985	2.264	0.201	0.513

MAS- Months after storage
Values in parenthesis are square root transferred values

The values in the same column with the same alphabet are not significantly different

In the field study, a progressive decrease in the germination percentage was observed with increasing doses of gamma rays. The treatment (T_5) 500 Gy exhibited a low germination percentage of 62% followed by (T_4) 400 Gy with 76 %, and (T_3) 300 Gy with 84 %. Treatment 100 Gy and 200 Gy showed no difference in germination percentage compared to control. Higher gamma doses ranging from 350-500 Gy decreased germination percentage of chickpea (Hameed *et al.*, 2008). Highest dose of gamma rays 500 Gy (T_5) resulted in reduction in plant height (35.99cm). There was no significant difference among the mean values of control, 100 Gy, 200 and 300 Gy The mean number of pods $plant^{-1}$ ranged between 15.20 nos in (T_5) 500 Gy to 16.67 nos in (T_2) 200 Gy as compared to the (T_6) control (16.37 nos). The highest mean was recorded in (T_2) 200 Gy with 16.67 nos and the lowest in (T_5) 500 Gy with 15.20 nos. Gamma rays treated populations exhibited lower treatment mean for number of seeds pod^{-1} as compared to control at higher doses. The mean for this character was highest in (T_3) 300 Gy (15.27 nos), followed by (T_2) 200 Gy (15.25 nos) and lowest in treatment (T_5) 500 Gy (15.08 nos) among all the treatments.

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Table 4: Field evaluation of gamma irradiated seeds for morphological parameters

Treatment	Germination percentage (%)	Plant height (cm)	Number of pods $plant^{-1}$	Number of seeds pod^{-1}	100 seed test weight (g)
T_1 (100 Gy)	96.0[a] (9.849)	39.92[a] (6.397)	16.48[b] (4.181)	15.24[a] (4.030)	15.20[a] (4.025)
T_2 (200 Gy)	96.0[a] (9.849)	39.91[a] (6.396)	16.67[a] (4.204)	15.25[a] (4.031)	10.53 (3.396)
T_3 (300 Gy)	84.0[b] (9.218)	39.96[a] (6.400)	16.39[b] (4.170)	15.27[a] (4.034)	10.52 (3.395)
T_4 (400 Gy)	76.0[c] (8.774)	38.52[b] (6.287)	16.39[b] (4.170)	15.10[b] (4.012)	10.49 (3.390)
T_5 (500 Gy)	62.0[d] (7.937)	35.99[b] (6.082)	15.20[d] (4.025)	15.08[c] (4.010)	10.48 (3.388)
T_6 (Control)	96.0[a] (9.849)	39.95[c] (6.399)	16.37[b] (4.168)	15.20[a] (4.025)	10.52 (3.395)
SE (m)	0.077	0.003	0.004	0.003	0.003
CD (5%)	0.245	0.009	0.013	0.009	NS

NS- Non significant **MAS- Months after storage**
Values in parenthesis are square root transferred values
The values in the same column with the same alphabet are not significantly different

Less variation in means of 100 seed weight was observed in all the treatments and there were no significant differences among the treatments. Higher means for this character were recorded in (T_2) 200 Gy compared to control. An increase in 1000 kernel weight and harvest index of canola was found in seeds treated with 100 Gy gamma rays (Rahimi and Bahrani, 2011).

Sometimes the reduction in physiological traits may be due to sudden destruction of growth inhibitors and metabolic changes (Ariraman *et al.*, 2014). No morphological abnormalities was observed in control and lower doses of gamma rays , whereas few crinkled leaves were found in higher doses like (T_4) 400 Gy and (T_5) 500 Gy in the early days. However, these plants recovered and produced normal leaflets afterwards.

CONCLUSION

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Gamma radiation at 200 Gy registered a higher value for seed germination parameters and was highly effective in controlling pulse beetle infestation. Though, gamma doses above 200 Gy were effective in controlling pulse beetle, it showed reduction in germination parameters and morphological traits.

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