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Studies on effectiveness and efficiency of gamma rays on cowpea in M_2 generation

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Abstract
Cowpea is one of the most important food crops and it's the ability to grow in a wide range of climate conditions. The aim of the investigation to assess the mutagenic effectiveness and efficiency of gamma rays in the M_2 generation of cowpea. The seeds were treated with different doses of gamma irradiation such as 200, 400, 600, 800, 1000, and 1200 Gy. The biological damage is a measure based on the lethality and injury of the M_1 generation. Mutants were recorded in M_2 generation and to calculate the effectiveness and efficiency of gamma rays. The present results revealed that both mutagenic effectiveness and efficiency were higher at lower doses of irradiation. In 200 Gy and 400 Gy, doses showed better mutagenic effectiveness and efficiency than the other irradiation doses. Hence, gamma irradiation induces a high impact in lower doses and less biological damage in higher doses.

Keywords: cowpea, gamma irradiation, effectiveness, efficiency, biological damage

Introduction
Cowpea (*Vigna unguiculata* (L.) Walp.) is an important food legume crop and rich in nutrition, consumed by humans and livestock. It is a tropical grain legume plant that played an important role in developing countries and a good source of protein content, rich in amino acids (Elhardallou *et al*., 2015) [1]. Genetic variability is significant for crop development, but in self-pollinating plants like cowpea is a lack of genetic variability due to natural selection. Induced mutations provide a sign of genetic variability: therefore, mutation breeding used and played a considerable role in many crops (Vanmathi *et al*., 2021) [2].Mutation breeding plays a significant role in producing desired beneficial mutations done in many different crops across the globe level and more than 3,365 crop varieties have been released for commercialization with resistance to stress and improvement of agronomic traits (IAEA, 2019; Oladosu *et al*., 2016) [3, 4]. Induced mutagenesis has played a crucial role in developing a new variety by improving particular characters without modifying the genetic content (Raina et al., 2016) [5]. The previous research reported that the mutation breeding was developed in many techniques, but gamma-ray is the most effective mutagen (Bae *et al*., 2012; Okunola *et al*., 2020) [6, 7]. Effectiveness means the ability of mutagens to induce the desired mutations and its measure to mutation rate relative to dose or concentration (Awnindra *et al*., 2001) [8]. Efficiency is an idea of the proportion of mutations concerning other associated biological effects such as injury, lethality, sterility induced by mutagen (Kousar and Babu, 2010) [9]. The physiological damage is caused by mutagens which are generally correlated with the frequency of mutations and used as analysis effectiveness of the mutagen (Kume and Todoriki, 2013; Sadecka, 2007) [10, 11]. Since, any mutation breeding programme, familiarity of relative biological effectiveness and efficiency of different mutagens and their selection of high frequency of essential mutations. Hence, the present experiment was to determine the effectiveness and efficiency of the mutagen and select the optimum doses of gamma irradiation.

Materials and methods
One of the varieties of cowpea (CO7) seeds was collected from National Pulse Research Centre (NPRC), Vamban, Pudukkottai, Tamil Nadu, India.

Raising M1 generation
Uniformly, matured and healthy seeds were treated with different doses of gamma irradiation such as 200, 400, 600, 8000, 1000, and 1200 Gy with a source of cobalt (^{60}CO) by Indira Gandhi Centre for Atomic Research (IGCAR), Tamil Nadu, India. After the completion of irradiation, seeds were sown in the field along with the control in a randomized block design with three replications during 2016 for raising M_1 generation. All the cultural practices such as plant protection, irrigation, and removal of weeds were carried out during the plant's growth till the plant's harvesting stage. All the treatments, including control, have adopted a spacing of 45 cm between rows and 30 cm between plants to plant. After the maturation of M_1 plants, the seeds were collected from the respective dose of mutagens to raise M_2 generation.

Raising M_2 generation
After the completion of M_1 generation, the seeds were to raise the M_2 generation randomly selected healthy seeds harvested from the M_1 generation of each treatment with randomized block design with three replications during 2017.

Mutation frequency, mutagenic effectiveness and efficiency, and mutation rate

Mutation frequency, mutagenic effectiveness, and efficiency were calculated by Konzak *et al*., 1965 [12] and expressed as a percentage.

Mutation frequency

Mutation frequency measured as the number of mutants was expressed in the M_2 generation and conveyed as a percentage.

$$\text{Mutation frequency (\%)} = \frac{\text{Number of mutants}}{\text{Total number of } M_2 \text{ plant}} \text{ X } 100$$

Mutagenic effectiveness

Mutagenic effectiveness is used to determine the frequency of mutations induced by a dose of particular mutagens. The following formula can be used to calculate the effectiveness of the individual doses of gamma irradiation.

$$\text{Mutagenic effectiveness} = \frac{\text{Mutation frequency (\%)}}{\text{Dose in Gray (Gy)}}$$

Mutagenic efficiency

Mutagenic efficiency represents the proportion of mutations in biological damage such as lethality, injury, sterility, etc,

$$\text{Mutagenic efficiency} = \text{Mutation frequency/ Injury}$$
$$= \text{Mutation frequency /Lethality}$$

Whereas, 'Injury' was calculated as percentage of reduction of plant height and 'Lethality' was represented as percentage of survival reduction.

Mutation rate (MR)

The mutation rate is calculated to the ability of mutations induced by specific mutagens of doses/ concentrations of the mutagens and measured by the following formula.

$$\text{Mutation rate} = \frac{\text{Sum of values of efficiency or effectiveness of particular mutagen}}{\text{Number of treatments of a particular mutagen}}$$

Table 1: Mutagenic effectiveness and efficiency of gamma rays in *Vigna unguiculata* (L.) Walp. in M_2 generation

Gamma rays (Gy)	Mutation frequency (%)	(Lethality) (%)	(Injury) (%)	Effectiveness MF/Gy	Efficiency	
					L=MF/L	I=MF/I
200 Gy	4.54	18.81	11.76	0.0227	0.241	0.386
400 Gy	8.04	27.05	17.64	0.0201	0.297	0.549
600 Gy	4.16	35.29	25.87	0.0069	0.117	0.160
800 Gy	2.4	43.53	30.58	0.003	0.055	0.078

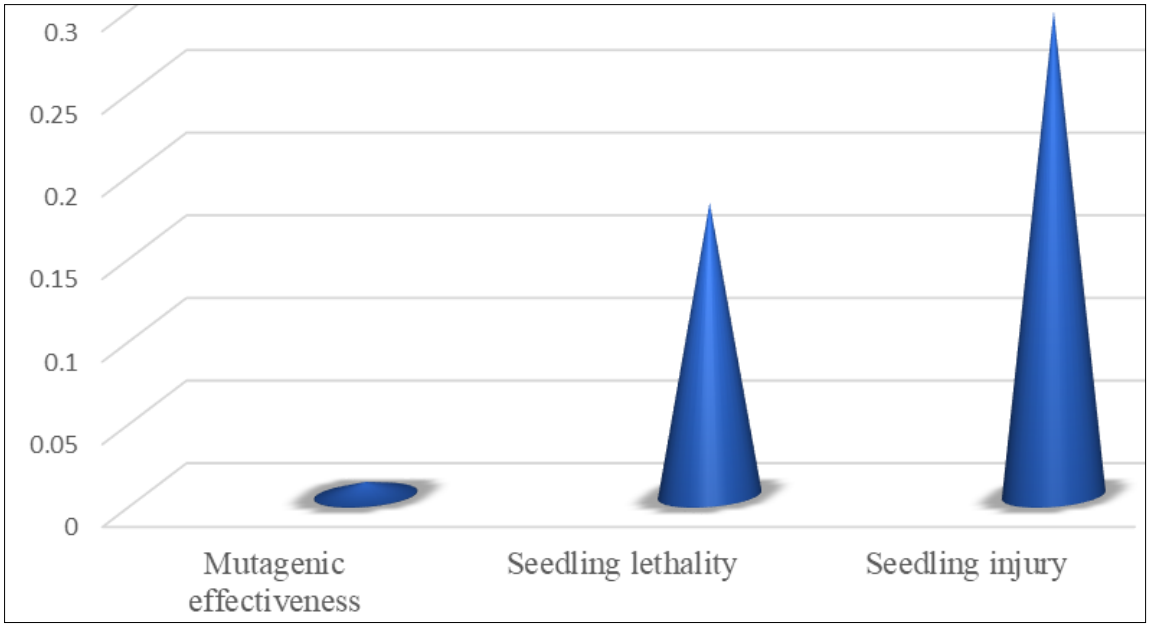

Fig 1: Mutation rate of mutagens in mutagenic effectiveness and biological damage of seedling injury and seedling lethality of cowpea.

Results

The mutagenic effectiveness was measured based on the number of mutations per unit of doses of mutagen. The effectiveness range from 0.0227 to 0.003 of the gamma rays treatments (Table 1). Mutagenic effectiveness was higher at increasing doses of gamma rays. Among the different doses of gamma irradiation, the maximum effectiveness was found in 200 Gy and followed by 400 Gy, 600 Gy, and 800 Gy. The mutagenic effectiveness was more effective in lower doses of gamma rays. In the present study, the increasing doses of the mutagenic effectiveness were declined because the mutagen induced was most effective.

Mutagenic efficiency was calculated based on biological damages such as seedling injury (MF/I), seedling lethality (MF/L). Measure the mutagenic efficiency not only the biological damage but also depends upon the physical wounds and other stresses. The efficiency of gamma radiations declined considerably with increasing doses of mutagens. The seedling lethality 400 Gy showed was most efficient among the other mutagenic treatments. At the same

time seedling injury, 400 Gy of gamma rays was most efficient than the other doses of gamma irradiation (Table. 1). The seedling injury and lethality expressed the efficiency of mutagens in the following order 400 Gy > 200 Gy > 600 Gy > 800 Gy. The present investigation exposed the moderate doses that were most efficient compared to other doses.

The mutation rate was measured, the effectiveness and efficiency of the mutagens, and also provides knowledge about the average rate of mutation induction per mutagen. The mutation rate is calculated based on the mutagenic effectiveness and biological damage such as lethality and injury. The mutation rate of seedling lethality was 0.177, and the injury value was 0.293. While the mutagen rate of effectiveness was 0.008, it represents the impact of the gamma irradiation (Fig.1).

Discussion

The usefulness of mutagenesis in plant breeding techniques depends not only on effectiveness but also on the efficiency of mutagen to reclaim the frequency of mutations (Siddique and Ansari, 2005) [13]. The present experiment shows the mutagenic effectiveness and efficiency were higher in lower doses of gamma irradiation. Mutagenic effectiveness and efficiency are important in mutation breeding to select the optimum dose or concentration of the mutagens. This finding research, the report shows mutagenic effectiveness were declined in higher doses, it indicating the increase the strength of mutation rate, and many authors reported in finger millet (Muduli and Misra, 2007) [14], and black gram (Arvind kumar *et al*., 2007) [15].

Mutagenic efficiency was high in lower or moderate doses of gamma irradiation. A similar finding was reported in *Vigna unguiculata* L. Walp. (Dhanavel *et al*., 2008) [16], and black gram (Sharma *et al*., 2005) [17]. The frequency and spectrum of chlorophyll and morphological mutations is another way to determine the effects of mutagenic treatments. Mutagenic effectiveness is an index of the response of a genotype to the increasing doses of the mutagen. Mutagenic efficiency indicates the extent of genetic damage recorded in the M_2 generation relative to the biological damage caused in the M_1 generation (Khan *et al*., 2009; Wani, 2009) [18, 19].

In our study, the lower and intermediate dose of gamma rays was more efficient because it caused less biological damage and produced a desirable mutation. In mutation breeding, mutagenic effectiveness and efficiency are essential for selecting the desirable traits. The current experiment revealed that the mutagenic effectiveness and efficiency was declined in higher doses of gamma irradiation and the similar points reported in finger millet under the effect of gamma irradiation (Ambavane *et al*., 2015) [20]. Many authors have been reported that the effectiveness and efficiency of mutagenesis studied in many plants like lima bean (Kumar *et al*., 2003) [21], green gram (Velu *et al*., 2008) [22], lentil (Satpute, 2009; Laskar *et al*., 2018) [23, 24], mung bean (Wani *et al*., 2107) [25], and cluster bean (Shinde, 2013) [26] under individual treatment of gamma rays, chemical mutagens and combination of physical and chemical mutagens.

Conclusion

The present investigation is to study the effect of mutagenic effectiveness and efficiency of cowpea in M_2 generation. The current, work concluded the 200 Gy doses exposed are the most effective dose. The efficiency was showed 400 Gy is highly effective compared to other doses. Both mutagenic effectiveness and efficiency were high in lower doses and decreased in high doses of irradiation. Hence, lower or moderate doses of gamma irradiation are induced for future mutation breeding to gain the desirable traits for crop improvement.

Declaration

The authors declare no conflict of interest by the authors.

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