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# Median lethal dose estimation of physical and chemical mutagens in *Gloriosa superba* L.

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**Abstract**
Induced mutagenesis is an ingenious tool to improve the genetic fidelity of a crop through creation of variability especially in vegetatively propagated crops like glory lily. The current research was aimed to determine the lethal dose ($LD_{50}$) and the effect of physical mutagen (gamma rays) and chemical mutagens (Ethyl Methane Sulphonate (EMS)) and (Diethyl Sulphate (DES)) on germination percentage, plant height, number of leaves and tuber weight derived from mutagen treated seeds of *Gloriosa superba* cv. Andhra wild to create variability for desirable traits. Self-pollinated seeds were exposed to different doses of gamma rays using $^{60}Co$ gamma chamber at Sugarcane Breeding Institute, Coimbatore. Another set of pre-soaked seeds were treated with freshly prepared solution of EMS and DES. The treated seeds including control were sown in the nursery beds under shade net in completely randomized design (CRD) with 5 replications. The results revealed a gradual and significant reduction in germination percentage, plant height, number of leaves and individual tuber weight with increase in dosage of mutagens which had profound effect on the above variables due to seed injury resulting in poor growth of the seedlings. Based on the probit curve, LD50 dose for gamma rays, EMS and DES were 20.96 kR, 1.91% and 1.13% respectively. Higher doses of physical and chemical mutagens had negative effect on the morphological characteristics of the seedlings derived from mutagen treated seeds.

**Keywords:** Glory lily, physical and chemical mutagens, $LD_{50}$, germination percentage

## Introduction
*Gloriosa superba* commonly known as Kalahari (Hindi) glory lily, kanvazhipoo, kanvazhikizhangu, senkandhal, karthigaikizhangu (Tamil) is an export oriented medicinal plant. It is cultivated in Tamil Nadu mainly in Tirupur, Din Digul and Karur districts of Tamil Nadu in an area of 3000 ha. The annual production and export of Gloriosa seeds from the state is around 600 tonnes with a foreign exchange of Rs. 120 crores. Ever since the crop was commercialized, it attracted the attention of many traders, buyers, sellers, agents, brokers, importers and processors. The cultivation of *Gloriosa superba* gained momentum when its price touched Rs.1500/kg during 2007 and the farmers started intensive farming by adopting various improved package of practices. An estimate on the production reports that 700-1000 ton of dry seeds are produced in Tamil Nadu, Karnataka and Andhra Pradesh, now extending upto Maharastra, Madhya Pradesh and Orissa. Glory lily seeds are exported to Europe, mainly Italy, Hungary besides countries like Germany and USA fetching a foreign exchange of Rs.120 crores every year.

*Gloriosa superb*a is a perennial herbaceous climber growing up to 3.5 to 6.0 metres in length. The climber is trained at 1-2 metres above the ground level. The vines grow tall, very thin, climbing nature and are weak stemmed with 'V' shaped tuberous roots.

The plant has been used in Indian system of medicine since time immemorial. Though it is poisonous, it is used as anti-periodic, tonic, Anthelmintic and also against snake bites and scorpion sting. It is sometimes used for promoting labour pain and also as abortifacient. It is considered useful in colic, chronic ulcers, piles and gonorrhoea. The medicinal properties of the drug are due to the presence of alkaloids, chiefly 'colchicine' ($CH_{22}O_{25}N_6$) and 'gloriosine' ($CH_{22}O_{25}N_6$). Colchicine is used in the treatment of 'Gout', a common disorder in temperate countries exploited but after knowing the fact that the seeds have higher quantity of alkaloids, the crop is grown now mainly for seeds which are in good demand. Because of the various medicinal properties the demand is escalating continuously every year. (Nadkarni, 1996) [18]

*Gloriosa* being a highly cross pollinated, clonally propagated crop, any intervention on crop improvement could be possible either through hybridization or by mutation. Mutation breeding has been successfully used for generating genetic variation and breeding new varieties in many crops during the past few decades (Van Harten, 1998; Tambe and Apparao, 2009) [16, 15] and become ultimate source of genetic variation to provide unique germplasm and the raw material for plant breeders.

## Materials and methods

Self-pollinated, genetically pure seeds of cultivar Andhra Wild were chosen for induction of mutation. The first grade seeds were initially treated with hot water soaking for 30min followed by overnight drenching, decanted and shade dried. The mutagens included, gamma rays ($^{60}$Co) (physical mutagen), Ethyl Methane Sulphonate (EMS) and Diethyl Sulphate (DES) (chemical mutagen) for induced mutagenesis. One hundred seeds of cv Andhra Wild were exposed to different doses (10 kR, 15 kR, 20kR, 25 kR, 30 kR, 35 kR, 40 kR and 45 kR) of gamma rays using $^{60}$Co gamma chamber at Sugarcane Breeding Institute, Coimbatore. For chemical mutagenesis, 100 seeds each were treated in fresh solution of EMS (1%, 1.5%, 2%, 2.5%, 3%. 3.5%, 4% and 4.5%) and DES (0.5%, 0.75%, 1%, 1.25%, 1.5%, 1.75%, 2% and 2.25%) including control was prepared in phosphate buffer at pH 7.0 and the treatment was given for 30 min with intermittent shaking. Finally, the treated seeds were rinsed in running tap water thoroughly to remove the chemical residues. The mutagen treated seeds were sown in raised nursery beds along with control in completely randomized design (CRD) with 5 replications during 2016-17 at the Department of Medicinal and Aromatic Crops, TNAU, Coimbatore. The $LD_{50}$ value was calculated based on probit analysis using the germination of treated seeds to that of control.

## Probit analysis

The $LD_{50}$ value both physical and chemical mutagens were determined based on the probit analysis (Finney, 1978) [4]. The probit function is the inverse cumulative distribution function (CDF) or quantile function associated with the standard normal distribution.

## Analysis of variance

Data were subjected to the standard analysis of variance procedure using SPSS statistical package to identify the lethal dose ($LD_{50}$). The $LD_{50}$ for each mutagen was estimated through the simple linear regression model by fitting the straight line equation y=a + bx; where y is the response variable (percent survival), x is the independent variable (physical/ chemical mutagen), while and b represent the slope and constant, respectively.

Further data was recorded on morphological parameters viz., plant height, number of leaves and tuber weight of the survived mutants.

## Results and discussion

### Determination of lethal dose

40-60% of survival rate (Van Harten, 1998) [16] and 30-50% of growth reduction in $M_1$ seedlings has often been used as a criterion for a promising treatment for mutagen treatment. In the present study, a gradual reduction in germination of the seedlings was observed with increase in dosage of physical and chemical mutagens. The $LD_{50}$ for gamma rays and chemical mutagens were fixed based on the critical dosage of mutagens causing 50 percent mortality of the seedlings using probit analysis. The germination percentage ranged from 2 to 96% (gamma rays), 4 to 94% (EMS) and 8 to 98% (DES) in different doses of mutagens (Table 1). $LD_{50}$ value for gamma rays, EMS and DES as assessed from probit curve analysis (Fig 1, 2 and 3) was 20.96 kR, 1.91% and 1.13% respectively. Previous studies on physical and chemical mutagens revealed that survival of plants to maturity depends on the nature and extent of chromosomal damage (Adamu and Aliyu, 2007; Khan and Goyal, 2009; Monica and Seetharaman, 2016) may be responsible for reduction in germination ability, plant growth and survival.

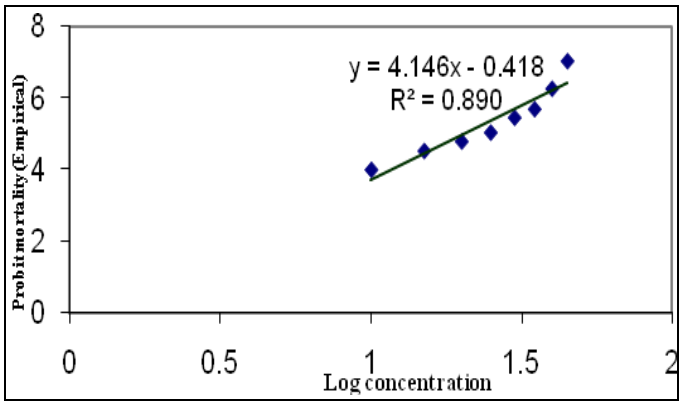


**Fig 1:** Probit analysis based on corrected mortality rates for gamma irradiation.

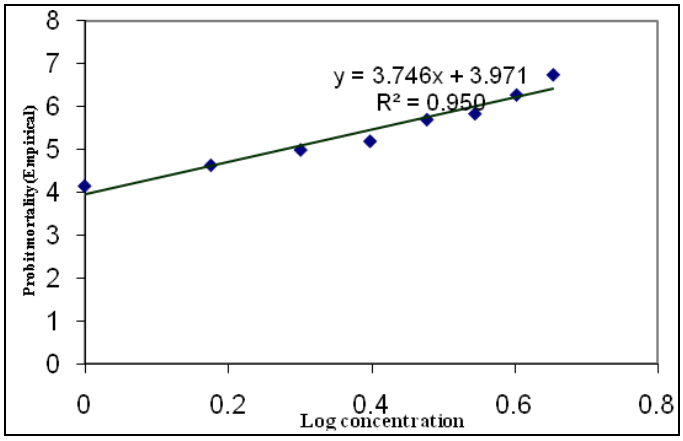


**Fig 2:** Probit analysis based on corrected mortality rates for Ethyl Methane Sulphonate (EMS).

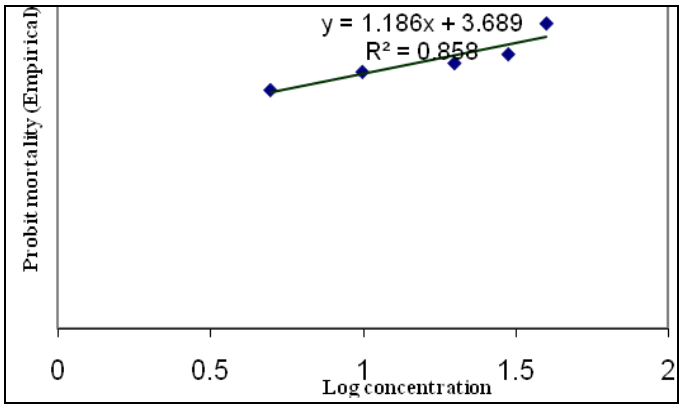


**Fig 3:** Probit analysis based on corrected mortality rates for Diethyl Sulphate (DES).

Reduced survival percent at higher doses of gamma radiation may be due to killing of cells and ionization of the nuclei. The higher level of dosages tried both in physical and chemical mutagens might have caused inhibition in mitosis on cell division and elongation due to inactivation and/or decrease in auxin content leading to poor establishment and survival (Vishwanathan *et al*., 1992) [17]. Similar results were observed by Mangayarkarasi *et al*. (2014) [10] and Kannabiran *et al*. (2017) [8] in periwinkle, Jayakumar and Selvaraj (2003) [7] in sunflower and Jabee and Ansari (2005) [6] in chickpea.

**Table 1:** Effect of mutagens (gamma radiation, EMS and DES) on germination of *Gloriosa superba* cv. Andhra Wild

| Gamma irradiation | | | | Ethyl methane sulphonate (EMS) | | | | Diethyl sulphate (DES) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dose (kR) | Germination percentage (%) | Percent germination over control (%) | Percent germination reduction over control (%) | Dose (%) | Germination percentage (%) | Percent germination over control (%) | Percent germination reduction over control (%) | Dose (%) | Germination percentage (%) | Percent germination over control (%) | Percent germination reduction over control (%) |
| 0 | 96 | 100.00 | 0.00 | 0.0 | 94 | 100.00 | 0.00 | 0 | 98 | 100.00 | 0.00 |
| 10 | 84 | 87.50 | 12.50 | 1.0 | 80 | 85.11 | 14.89 | 0.50 | 86 | 87.76 | 12.24 |
| 15 | 68 | 70.83 | 29.17 | 1.5 | 64 | 68.09 | 31.91 | 0.75 | 72 | 73.47 | 26.53 |
| 20 | 58 | 60.42 | 39.58 | 2.0 | 50 | 53.19 | 46.81 | 1.00 | 60 | 61.22 | 38.78 |
| 25 | 48 | 50.00 | 50.00 | 2.5 | 42 | 44.68 | 55.32 | 1.25 | 54 | 55.10 | 44.90 |
| 30 | 32 | 33.33 | 66.67 | 3.0 | 24 | 25.53 | 74.47 | 1.50 | 36 | 36.73 | 63.27 |
| 35 | 24 | 25.00 | 75.00 | 3.5 | 20 | 21.28 | 78.72 | 1.75 | 24 | 24.49 | 75.51 |
| 40 | 10 | 10.42 | 89.58 | 4.0 | 10 | 10.64 | 89.36 | 2.00 | 18 | 18.37 | 81.63 |
| 45 | 2 | 2.08 | 97.92 | 4.5 | 4 | 4.26 | 95.74 | 2.25 | 8 | 8.16 | 91.84 |

**Impact of induced mutagenesis growth variables**

The results showed that differences among the different physical and chemical mutagens considerably influenced the seedling height, number of leaves and tuber weight. There was a inverse relationship between the dosage and various growth parameters (Table 3, 4 and 5). The mutagens, specifically the gamma rays was more potent and highly penetrating nature might have impacted the cells undergoing meiotic division in the bud region (Deshpande *et al*, 2010) [3]. The reduction in shoot length can be ascribed to delay in germination and slow growth rate in mutated seedlings. These results are in line with Gaul (1977) [5] who reported that increasing radiation doses on M1 generation resulted in decreasing plant height and root length. The decline in the growth parameters with increase in dosage of mutagens as experienced in the present study was earlier observed in periwinkle by Kannabiran *et al*. (2017) [8]. Similarly Arul doss and Mullainathan (2015) [2] showed a dose dependant decrease for most of the characters viz., plant height, primary and secondary branches per plant, number of leaves, days to first flowering, fruits per plant and dry fruit weight in gamma rays and EMS treated seeds in *Capsicum annuum*.

In tomato, Sikder *et al.* (2015) [14] revealed that seed germination, seedling height and pollen fertility in M1 generation reduced steadily with the increasing doses of both mutagens, gamma rays and EMS. Wi *et al*. (2007) [18] postulated that low dose of irradiation will induce growth stimulation by changing the hormonal signalling network in plant cells or by increasing the ant oxidative capacity of the cells. In contrast, the high dose treatment that caused growth inhibition has been ascribed to the cell cycle arrest at G2/M phase during somatic cell division and / or various damages in the entire genome. The fact that glory lily seedlings which were treated with lower doses of gamma rays and chemical mutagens grew better than those exposed to higher doses due to block in cellular DNA causing the plant growth to stop or slow down (Roslim *et al*., 2015) [13].

**Table 2:** Probit analysis for calculating $LD_{50}$ in *Gloriosa superba* cv. Andhra Wild

| Gamma irradiation | | | | | Ethyl methane sulphonate (EMS) | | | | | Diethyl sulphate (DES) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dose (kR) | Log10 of doses | Corrected mortality percentage | Empirical Probit unit | $LD_{50}$ value | Dose (%) | Log10 of doses | Corrected mortality percentage | Emprical probit unit | $LD_{50}$ value | Dose (%) | Log10 of doses | Corrected mortality percentage | Empirical Probit unit | $LD_{50}$ value |
| 0 | 0 | 4 | - | 20.96 | 0 | 0 | 6 | - | 1.91 | 0 | 0 | 2 | - | 1.13 |
| 10 | 1.00 | 16.00 | 4.01 | | 1.00 | 0.08 | 20 | 4.16 | | 0.50 | -0.30 | 14 | 3.92 | |
| 15 | 1.18 | 32.00 | 4.53 | | 1.50 | 0.18 | 36 | 4.64 | | 0.75 | -0.12 | 28 | 4.42 | |
| 20 | 1.30 | 42.00 | 4.80 | | 2.00 | 0.30 | 50 | 5.00 | | 1.00 | 0.04 | 40 | 4.75 | |
| 25 | 1.40 | 52.00 | 5.05 | | 2.50 | 0.40 | 58 | 5.20 | | 1.25 | 0.10 | 46 | 4.90 | |
| 30 | 1.48 | 68.00 | 5.47 | | 3.00 | 0.48 | 76 | 5.71 | | 1.50 | 0.18 | 64 | 5.36 | |
| 35 | 1.54 | 76.00 | 5.71 | | 3.50 | 0.54 | 80 | 5.84 | | 1.75 | 0.24 | 76 | 5.71 | |
| 40 | 1.60 | 90.00 | 6.28 | | 4.00 | 0.60 | 90 | 6.28 | | 2.00 | 0.30 | 82 | 5.92 | |

**Table 3:** Effect of gamma rays on seedling characters of *Gloriosa superba* cv. Andhra Wild

| Dose | Plant height (cm) | | | No. of leaves | | | Tuber weight | | |
|---|---|---|---|---|---|---|---|---|---|
| | Actual | Percent over control | Percent reduction over control | Actual | Percent over control | Percent reduction over control | Actual | Percent over control | Percent reduction over control |
| 0 | 10.4 | 100 | 0.00 | 4.5 | 100 | 0.00 | 1.88 | 100 | 0.00 |
| 10.00 | 8.5 | 81.73 | 18.27 | 4.2 | 93.33 | 6.67 | 1.75 | 93.09 | 6.91 |
| 15.00 | 7.2 | 69.23 | 30.77 | 4 | 88.88 | 11.12 | 1.54 | 81.91 | 18.09 |
| 20.00 | 6.5 | 62.5 | 37.5 | 3.7 | 82.22 | 17.78 | 1.4 | 74.47 | 25.53 |
| 25.00 | 6 | 57.69 | 42.69 | 3.5 | 77.77 | 22.23 | 1.36 | 72.34 | 27.66 |
| 30.00 | 4.8 | 46.15 | 53.85 | 3.2 | 71.11 | 28.89 | 1.03 | 54.79 | 45.21 |
| 35.00 | 3.7 | 35.58 | 64.42 | 3 | 66.66 | 33.34 | 0.9 | 47.87 | 52.13 |
| 40.00 | 3.1 | 29.81 | 70.19 | 2.4 | 53.33 | 46.67 | 0.85 | 45.21 | 54.79 |
| 45.00 | 3 | 28.85 | 71.15 | 2 | 44.44 | 55.56 | 0.74 | 39.36 | 60.64 |
| SEd | 0.196 | | | 0.207 | | | 0.031 | | |
| CD (0.5%) | 0.415 | | | 0.440 | | | 0.065 | | |

**Table 4:** Effect of Ethyl Methane Suphonate (EMS) on seedling characters of *Gloriosa superba* cv. Andhra Wild

| Dose (%) | Plant height (cm) | | | No. of leaves | | | Tuber weight | | |
|---|---|---|---|---|---|---|---|---|---|
| | Actual | Percent over control | Percent reduction over control | Actual | Percent over control | Percent reduction over control | Actual | Percent over control | Percent reduction over control |
| 0 | 11.2 | 100 | | 6.3 | 100 | | 2.36 | 100 | |
| 1.00 | 11 | 98.21 | 1.79 | 6 | 95.24 | 4.76 | 1.96 | 83.05 | 16.95 |
| 1.50 | 10.7 | 95.54 | 4.46 | 5.8 | 92.06 | 7.94 | 1.85 | 78.39 | 21.64 |
| 2.00 | 9.8 | 87.5 | 12.5 | 5.4 | 85.71 | 14.29 | 1.72 | 72.88 | 27.12 |
| 2.50 | 8.8 | 78.57 | 21.43 | 4.8 | 76.19 | 23.81 | 1.58 | 66.95 | 33.05 |
| 3.00 | 8 | 71.43 | 28.57 | 4.3 | 68.25 | 31.75 | 1.45 | 61.44 | 38.56 |
| 3.50 | 6.5 | 58.04 | 41.96 | 4 | 63.49 | 36.51 | 1.36 | 57.63 | 42.37 |
| 4.00 | 5.4 | 48.21 | 51.79 | 3.7 | 58.73 | 47.27 | 1.17 | 49.58 | 50.42 |
| 4.50 | 4.2 | 37.5 | 62.5 | 3.5 | 55.56 | 44.44 | 1.02 | 43.22 | 56.78 |
| SEd | 0.309 | | | 0.295 | | | 0.054 | | |
| CD (0.5%) | 0.654 | | | 0.625 | | | 0.114 | | |

**Table 5:** Effect of Diethyl Sulphate (DES) on seedling characters of *Gloriosa superba* cv. Andhra Wild

| Dose | Plant height (cm) | | | No. of leaves | | | Tuber weight | | |
|---|---|---|---|---|---|---|---|---|---|
| | Actual | Percent over control | Percent reduction over control | Actual | Percent over control | Percent reduction over control | Actual | Percent over control | Percent reduction over control |
| 0 | 11.0 | 100 | - | 5.5 | 100 | - | 2.08 | 100 | - |
| 0.50 | 10.8 | 98.18 | 1.82 | 5.4 | 98.18 | 1.82 | 2.00 | 96.15 | 3.85 |
| 0.75 | 10.3 | 95.37 | 4.63 | 5.1 | 92.73 | 7.27 | 1.88 | 90.38 | 9.62 |
| 1.00 | 9.8 | 89.09 | 10.91 | 4.6 | 83.64 | 16.36 | 1.65 | 79.33 | 20.67 |
| 1.25 | 8.5 | 77.27 | 22.73 | 4.2 | 76.36 | 23.64 | 1.60 | 76.92 | 23.08 |
| 1.50 | 7.4 | 67.27 | 32.73 | 4 | 72.73 | 27.27 | 1.47 | 70.67 | 29.33 |
| 1.75 | 6.5 | 59.09 | 40.91 | 3.9 | 70.91 | 29.09 | 1.34 | 64.42 | 35.58 |
| 2.00 | 4.8 | 43.64 | 56.36 | 3.8 | 69.09 | 30.91 | 1.20 | 57.69 | 42.31 |
| 2.25 | 3.5 | 31.82 | 68.18 | 3.8 | 69.09 | 30.91 | 1.00 | 48.08 | 51.92 |
| SEd | 0.238 | | | 0.148 | | | 2.393 | | |
| CD (0.5%) | 0.504 | | | 0.313 | | | 5.073 | | |

## Conclusion

Determination of $LD_{50}$ dose is an essential pre-requisite in mutation studies, as excess dosage leads to sterility or even lethality. For induction of desired mutations by radiation and chemical treatment being by chance, it is safer to choose the doses that can cause less damage and give higher multiplication and survival rates and also produce some useful mutations, which might be not possible by lower dosage. In the present study, based on the germination percentage of the seeds, the $LD_{50}$ dose for gamma irradiation, EMS and DES were 20.96 kR, 1.91% and 1.13% respectively. These optimal mutagen doses determined for glory lily could be useful while formulating mutation breeding programmes for improvement of economic traits such as increased seed yield and quality of the tubers.

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