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Effect of physical and chemical mutagens on total chlorophyll content of *Clitoria ternatea* l. in m$_1$ generation

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Abstract
Clitoria ternatea L. which is a medicinally and economically important plant belongs to Fabaceae family originated in tropical Asia. It is widely distributed throughout the humid, lowland tropics of Africa, Asia and Central America. The present investigation empirically evaluates the effects of physical and chemical mutagens on the evaluation of photosynthetic activity in terms of chlorophyll content. This article summarizes about the chlorophyll estimation and the experimental design with thirteen treatments with different concentrations of physical and chemical mutagens and the combination of both. The chlorophyll content is found to be highest in gamma irradiated plants followed by control plants. This work suggested that the physical mutagen helped C*litoria ternatea* L. plants enhancing the total chlorophyll content.

Keywords: Chlorophyll content, *Clitoria ternatea* L., EMS, gamma rays, mutation

1. Introduction
Mutagenesis is the process by which unanticipated familial changes take place in the genetic information of an organism not impelled by genetic isolation or genetic recombination, but brought forth by biological, physical and chemical mutagens [22]. Among the electromagnetic ionization radiation, more than α and β rays, γ-rays have high penetrating power [16]. EMS in particular, has been proven to be more efficient in the induction of mutations than radiations. Certain genes are mutated by radiations and not by EMS [6] and the mutation spectrum induced by the radiations and chemical mutagens is different [11, 3]. It was thought of interest to find the mutation frequencies when the physical and chemical mutagens were used in combination by many workers [14, 10, 12, 25]. Plant Mutation breeding triggers various types of mutagenesis. During induced mutagenesis, mutations come about as an exposure to irradiation (X-rays, γ-rays and ion beams, etc.,) or treatment with chemical mutagens; the process of starting off a mutation at a particular site in a DNA molecule is regarded as site-directed mutagenesis; and insertion mutagenesis is through DNA insertions, either through genetic transformation and insertion of T-DNA or activation of transposable elements [15, 8].

2. Materials and Methods
2.1 Plant materials and experimental site
The experiment was conducted in Botany department, SPPU, Pune - 411007. Genetically pure seeds of *Clitoria ternatea L.* blue variety known as *gokarn* in Marathi selected for further experiment were collected from Rahuri Krushi Vidyapeeth, Rahuri, Dist- Ahmednagar, Maharashtra, India.

2.2 Mutagens used for experiments
Gamma rays used as physical mutagen; Ethyl Methanesulphonate (EMS) used as a chemical mutagen and Combination of Gamma rays and EMS.

2.2.1 Gamma Rays Treatment
Healthy, uniform size and dry seeds of *Clitoria ternatea* L. blue flower variety were packed in the polythene bags and sealed for Gamma radiation. Electromagnetic ionizing radiations were applied from CO^{60} source of irradiation. Gamma radiation was carried out at Nuclear Chemistry Division, Department of Chemistry, Savitribai Phule Pune University, Ganeshkhind, Pune - 411007. The seed samples were exposed to *240Gy*, *300Gy*, *360Gy* and *420Gy* doses of γ-radiation.

2.2.2 EMS Treatment
To determine the lethal doses of LD_{50} at suitable concentrations of mutagens, Ethyl Methanesulphonate (EMS) was acquired from Spectrochem Pvt. Ltd. Mumbai (India) with a molecular weight of 124.16 g/mol and its density was 1.20 g/cm^3. Just about 25±2 ^{0}C temperature was maintained for EMS treatments. Dry, uniform size and healthy seeds (500) of the *Clitoria ternatea* L. blue flower varieties were selected for the present treatment. Different Concentrations of *0.25%, 0.50%, 0.75%* and 1*% of EMS* were selected for chemical mutagenic treatments.

2.2.3 Combination Treatment
For the combined treatment, γ-rays irradiated seeds of different doses of *240Gy; 300Gy*, *360Gy* and *420Gy* were used. After the physical mutagenic treatment, chemical mutagenic treatment of EMS was conducted on the seed samples. In combination treatment, γ-rays and EMS mutagens are combined as *240Gy+1%, 300Gy+0.75%, 360Gy+0.50%*, and *420Gy+0.25%.*

2.3 Statistical Data analysis
The experiment was set with three duplicates in accordance with RBD and the data was indicated as mean value of

Three duplicates. The data collected were checked out for invariance with one-way ANOVA and then by Duncan's multiple new range test (DMRT) at $p \le 0.05$ level. The Standard edition of SPSS version 26 programmed for Microsoft windows 10 was used with the help of Microsoft Excel for statistical error analysis.

2.4 Chlorophyll Estimation

Chlorophyll a, Chlorophyll b and the total chlorophyll content of *Clitoria ternatea* L. was estimated according to the formulation of (Arnon, 1949) per gram tissues as followed:

Chlorophyll *a* $= 12.7(A_{663}) – 2.69(A_{645}) \times \frac{v}{100 \times W}$ (mg g^{-1} fw)

Chlorophyll *b* $= 22.9(A_{645}) – 4.68(A_{663}) \times \frac{v}{100 \times W}$ (mg g^{-1} fw)

Total chlorophyll $= 20.2(A_{645}) – 8.02(A_{663}) \times \frac{v}{100 \times W}$ (mg g^{-1} fw)

Where V = Chlorophyll extract's final volume in 80% acetone in milliliters;
W= Fresh weight of tissue extracted in grams;
A = Absorbance at specific wavelength in nanometers;

3. Experimental Observations

The chlorophyll is essential component for photosynthesis. Total chlorophyll content was estimated in control, physical and chemical treated plants. In Control plants, the chlorophyll content was observed to be 25.88 mg g^{-1}. The estimated values in all treated plants were 11.30 to 29.97 mg g^{-1}. The maximum amount of chlorophyll content 29.97 mg g^{-1} was recorded in 240Gy treatments and the minimum amount of 11.30 mg g^{-1} was recorded in 420Gy+0.25% EMS treated plants.

4. Results and Discussion

The Chlorophyll content in leaves of a plant is a sign of its health [19]. *Chlorophyll a* and *Chlorophyll b* are vital pigments of the plant's photo-systems [22]. Furthermore, the *chlorophyll a* is the primary photosynthetic pigment in plants which helps producing energy in plants [24]. Yet it's empirically observed that *Chlorophyll b* concentration is 2-3 lesser than that of primary *Chlorophyll a* in plants [24]. Both in γ-irradiated and EMS treated seedlings, a little higher amount of *Chlorophyll a* than *Chlorophyll b* was observed in this investigation. This reduction in chlorophyll b pigments is caused by the disturbances in biosynthesis [16]. Gamma radiation induces various physiological and biochemical alteration in plants. The total chlorophyll content in *Clitoria ternatea* L. was enhanced (29.97 mg g^{-1}) in gamma treated plants of 240Gy followed by 21.08 mg g^{-1}, 18.46 mg g^{-1}, 15.57 mg g^{-1}. And the least value of chlorophyll content was observed in combination of gamma and EMS of 420+0.25%. In the present investigation, the chlorophyll contents of irradiated plants disclosed the highest value at the lowest dose of γ-rays. On account of the activated enzyme system, the amount of chlorophyll content raised on exposure to the lower doses of γ-rays (5). This would have intensified photosynthetic rate and hence increased yield in the field. Many experimental studies from the previous literature by [2, 4, 1, 9, 20, 18], showed the increased rate of photosynthesis and glucose content from γ-irradiated plants at lower doses. Those studies also had reported the improvement of yield components and Carbon containing compounds (chlorophyll parameters and carotenoid) in various plants such as tomato, maize, rice and wheat upon γ-irradiation. Higher dosage of γ irradiation had disclosed low chlorophyll content. And the effects of γ irradiation doses on chlorophyll was evaluated in terms of leaf gas-exchange, enzyme activity, disturbed hormonal balance and water exchange [25]. The γ-rays induced enlargement of thylakoid and changes in the metabolism and the site of metabolism were investigated by [27]. It is generally believed that gamma rays cause a reduction in growth of the plant but according to [21] gamma rays were found to be more effective in plant growth and chromosomal studies.

5. Tables and Figures

Mutagens	Concentration/ Dose	Chlorophyll *a* (*mg g*$^{-1}$)	Chlorophyll *b* (*mg g*$^{-1}$)	Total Chlorophyll (*mg g*$^{-1}$)
Control		16.22	9.66	25.88
EMS	0.25%	12.32	9.79	22.11
	0.50%	10.59	8.53	19.17
	0.75%	8.14	7.80	15.94
	1%	7.22	6.45	13.67
γ - Rays	240Gy	17.77	12.20	**29.97**
	300Gy	12.87	8.21	21.08
	360Gy	11.14	7.32	18.46
	420Gy	8.91	6.66	15.57
γ - Rays + EMS	240Gy+1%	11.41	9.38	20.79
	300Gy+0.75%	9.25	7.95	17.20
	360Gy+0.50%	7.49	7.12	14.61
	420Gy+0.25%	6.16	5.14	11.30

Fig 1: Effect of Mutagens on Total Chlorophyll content in M_1 Generation of *Clitoria ternatea* L.

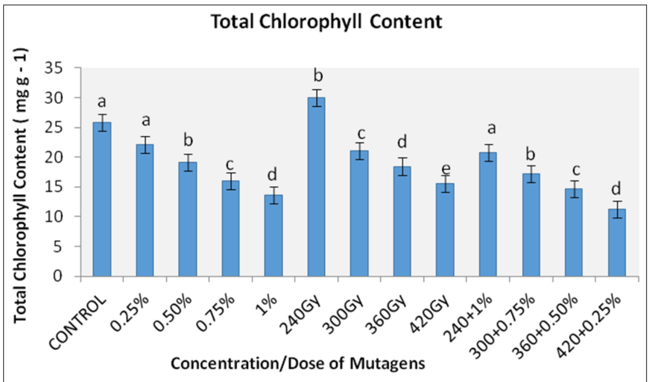

Fig 1: Effect of mutagens on Total Chlorophyll Content in M1 generation of *Clitoria Ternatea* L.

6. Conclusions

Compared to EMS treated plants, γ irradiated plants showed the enhanced content of Chlorophyll in M_1 generation of *Clitoria ternatea* L. And the increase in concentration or does mutagen showed the decreased content of Chlorophyll. The empirical results from the current experiment clearly indicate that 240Gy of gamma treated plants produced highest chlorophyll content. And lower dose of gamma radiation induced positive impact on *Clitoria ternatea* L. by enhancing the larger Chlorophyll pigments that gave their green color, and was an essential component of photosynthesis whereby they derive their energy for metabolism, growth, and reproductive processes.

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