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Induced Mutagenic Effect of Chemical and Physical Mutagens on Seedling Height, Seedling Injury, and Morphological Variations in Vigna Radiata (L). Wilczek

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Abstract:
Present study was conducted at the PDEA's Waghire College, Saswad, with fieldwork conducted in Village Hargude, Purandar, and laboratory work conducted at the Department of Botany. In order to investigate seedling height, seedling injury, and morphological variations, the M1 generation of Mungbean *Vigna radiata* (L) Wilczek variety Phule Chetak (PM-707-5) was cultivated using treated seeds for both physical and chemical mutagens, Gamma Radiation and Ethyl Methane Sulphonate (EMS). The seeds in the Phule Chetak PM-707 variety were subjected to physical mutagens with gamma radiation treatments of 100Gy, 150Gy, 200Gy, 250Gy, 300Gy, 350Gy, 400Gy, and 450Gy, as well as chemical mutagens, Ethyl Methane Sulphonate (EMS) at concentrations of 10mM, 20mM, 30mM, and 40mM. As the doses/Concentration increase, Seedling Height decreases, and Seedling Injury increases. Morphological variations observed in Leaf, Pod, and seed morphological characteristics. The normal leaf of the variety has Trifoliate leaves, but due to the effect of the mutagens, Bifoliate, Tetrafoliate, and Pentafoliate leaf variations have been observed. The variations in leaf lamina and apex, like the Lunate leaf, oblong leaf, Emerginate leaf apex, Bifurcated leaf apex, Dark green leaf reported in the different concentrations and doses of the mutagens.

Keywords: EMS, Gamma Radiation, Phule Chetak PM-707-5, Mutation, Mutagen.

Introduction:

In human nutrition, pulses play very vital role. Pulses contain amino acids and protein; consequently, pulses were also nicknamed "poor man's meat" (Kumar *et al*., 2010). Pulses are secondary to cereals in production and consumption in India. (ICMR) The Indian Council of Medical Research recommends a minimum consumption of protein of 47 g/day and the World Health Organization (WHO) recommends a per capita consumption of pulses @80g/day. Mungbean (*Vigna radiata* (L) Wilczek) is an important diploid 2n=22 annual crop belonging to the Fabaceae Family, which is commercially extremely important. It is mostly cultivated for Food and Fodder use. It is an essential pulse crop grown in India due to its short life cycle length, and it is typically cultivated in dry and semi-arid locations. Different crop rotation strategies can be done due to the short duration nature, wider adaptability, low water need,

and photoinsensitivity of Mungbean. (*Anil et al*., 2022).

Mungbean is a protein-rich stable food, and it contains 24-28% proteins and high lysine (504mg/g). It is rich in amino acids, vitamins, and minerals, which meet the dietary needs of the vegetarian population (Akash *et al*,2020). Consumption of mungbeans has increased because of the rising population and perceived health benefits (Datta *et al*.,2012). Hence there is an immediate need to improve cultivars with high productivity to meet the current and future demands.

In India, the productivity of pulse crops, especially mungbean in particular, is not sufficient to meet all the domestic demands of the growing population. The yield of mungbean is very low because to crop is grown in a rainfed area with poor management practices. In Mutation breeding, for induced use of chemical and physical mutagens in crop improvement is broadly accepted by plant breeders. (Das *et al.*,2021).

In the current study, the effects of chemical and physical mutagens on *Vigna radiata* (L) seedling height, seedling injury, and morphological variations were investigated. Wilczek Phule Chetak PM-707-5, Variety.

Material and Methods:

Experimental Materials:

In the present study Mungbean variety Phule Chetak PM-707-5 was used. Seeds were obtained from the Oilseed Research Station, Jalgaon. This variety is well adapted to the agroclimatic conditions of Maharashtra, including the experimental site of study. The field trial was conducted during the month of July at Hargude (Yadavwasti), Purandar, Dist-Pune, Maharashtra (India). The field is located between Latitude-18.2334º and Longitude 74.073799º. The experiment was carried out at both sites i.e Lab experiment for seedling study and Field trial for study morphological variations. Laboratory treatment was done at the botany laboratory of PDEA's Waghire College, Saswad, and a field trial was carried out at Hargude (Yadavwasti), Purandar, Pune. The soil of the Field site was medium black in texture and with good drainage.

Table.1 Details of the selected variety

Name of Variety	Source	Distinguishing Characters
Phule Chetak PM-707-5	Oilseed Research Station, Jalgaon.	High yielding,resistance to major diseases,synchronous maturity

Chemical Mutagen:

The chemical mutagen Ethyl Methane Sulphonate (EMS) ($CH_3SO_3C_2H_5$, molecular weight 124.16, density D4 25=1.203 g/ml, boiling point 80/100 mm Hg) was employed to cause mutation. The procedure was performed at department of botany,Waghire College, Saswad.

Table.3: Dose level of EMS

Sr. No	Concentration of the solution (mM)	Total volume required (phosphate buffer) (ml)	Volume to be taken Ethyl Methane Sulphonate (EMS) (ml)
1	10mM	50	0.0522

2	20mM	50	0.1045
3	30mM	50	0.1566
4	40mM	50	0.2088

Physical Mutagen:

The seeds of the mungbean variety Phule chetak were exposed to gamma radiation, a physical mutagen. The gamma ray source used for the treatment was Cobalt-60 (60Co). After being wrapped in paper covers, the dry, uniform, and well-filled seeds were put in the gamma cell and subjected to gamma radiation for the proper amount of time. As a control, non-irradiated, uniform, healthy seeds were utilized.

Table.2: Dose level of gamma rays

Treatments	Dose (Gy)	Exposure Time
T_1	100Gy	4 min 20 sec
T_2	150Gy	6 min 20 sec
T_3	200Gy	8 min 40 sec
T_4	250Gy	10 min 40 sec
T_5	300Gy	13 min 0 sec
T_6	350Gy	15 min 20 sec
T_7	400Gy	17 min 20 sec
T_8	450Gy	19 min 40 sec

Laboratory Studies:

In order to undertake laboratory analysis, the treated seeds were inserted in the roll of germination paper. Control seeds were untreated and soaked in distilled water before.

Observations under laboratory conditions:

The results of the present investigation on "Induction of genetic variation in *Vigna radiata* (L) Wilczk by Chemical and physical mutagens". In the variety Phule Chetak.

The morphological measurements of seedling damage, root length (cm), and shoot length (cm) were recorded.

Root and Shoot length:

On the seventh day after seeding, ten seedlings from each treatment and control group were chosen at random to have their root and shoot lengths measured. The average results were entered into a table.

Seedling injury:

On the seventh day, seedling height was measured. Seedling injury was defined as a decrease in the mean length of the seedling relative to the control and was represented as a percentage. The following formula was used to determine the seedling injury:

$$\% \text{ seedling injury} = \frac{\text{Control seedling height} - \text{Treatment seedling height}}{\text{Control seedling height}} \times 100$$

Control seedling height

Observation Table:

Table.4 Effect of mutagens in M1 generation of Mungbean var. Phule Chetak PM-707-5

Treatments	No.of. Seeds placed for seedling study	Total number of seedlings studied	Shoot length (cm)	Root length (cm)	Ratio	Total Seedling Height (cm)	Seedling injury (%)
Control	100	10	10.00	2.87	0.33	12.87	00
100Gy	100	10	7.56	3.15	0.42	10.71	16.78
150Gy	100	10	8.99	3.74	0.41	12.73	1.08
200Gy	100	10	11.13	4.36	0.39	15.49	20.35
250Gy	100	10	7.24	3.28	0.45	10.52	18.25
300Gy	100	10	8.80	4.36	0.50	13.16	2.25
350Gy	100	10	7.19	4.44	0.61	11.63	9.63
400Gy	100	10	6.96	4.02	0.59	10.98	14.68
450Gy	100	10	5.63	3.30	0.61	8.93	30.61
Control	100	10	9.38	4.17	0.50	13.55	00
10mM	100	10	7.94	3.81	0.46	11.75	13.28
20mM	100	10	7.84	3.61	0.51	11.45	15.49
30mM	100	10	7.31	3.64	0.49	10.95	19.18
40mM	100	10	6.11	2.92	0.47	9.03	33.35

Field Studies:

Total 300 seeds per treatment were planted at a 30 cm row-to-row and 10 cm plant-to-plant spacing in the experimental field site.

Data Collection under field conditions:

The morphological leaf variations were observed in various plant growth stages.

Results and Discussion:

Effect of mutagens in M_1 generation seedlings under laboratory conditions

Total 100 seeds placed for seedling study and after 7^{th} day Root and Shoot height were recorded and seedling injury calculated by using recorded observations.

Effect of mutagens on shoot length

The mean shoot length of populations treated with EMS under laboratory conditions varied between 6.11 cm (40 mM) to 7.94 cm (10 mM) compared to the control (9.38 cm). Highest shoot length observed at 10mM and lowest at 40Mm Concentration.The average shoot length of the seedling in populations treated with gamma radiation varied between 5.63 cm (450 Gy) to 11.13 cm (200 Gy) compared to the control (10.00 cm).Highest observed at 200Gy and lowest at 450Gy.

Fig.1 Field view of the experimental plot

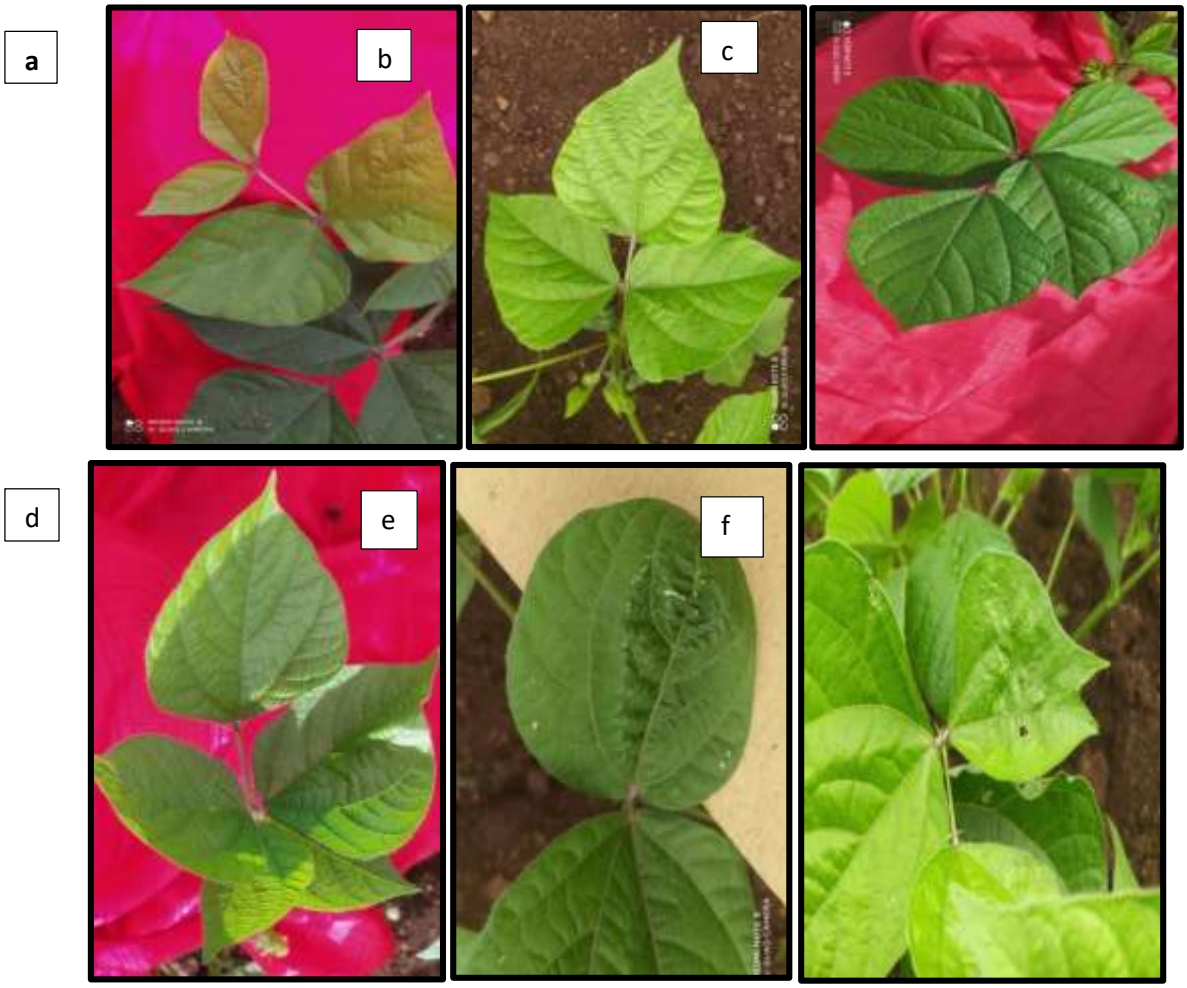

Fig.2: Leaf variations observed in the M_1 generation of Mungbean

1. Bifoliate leaves
2. Control (Trifoliate leaf)
3. Tetrafoliate leaves

4. Pentafoliate leaves
5. Lunate leaf f)Bifurcated apex

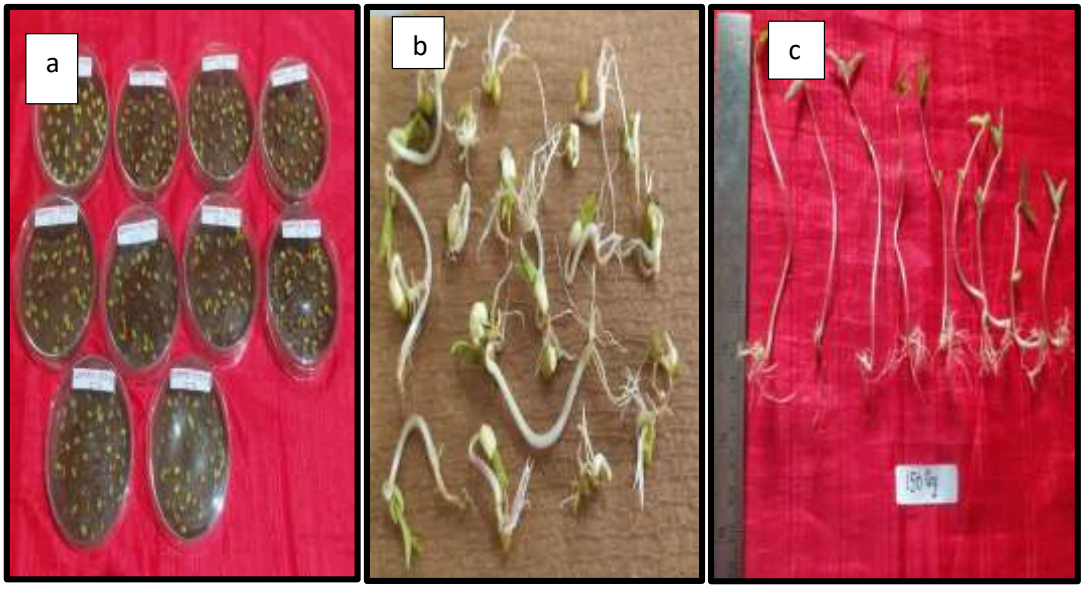

Fig 3: **Seeds placed for seedling study**
a)Treated seeds b)Plumule and Radicle emergence c) Seedlings

Effect of mutagen on root length

In EMS-treated populations mean root length of the seedling ranged from 2.92 cm (40mM) to 3.81cm (10mM) over the control (4.17 cm).Highest root length in EMS treated seeds observed at 10mM and lowest at 40mM. In Gamma Radiation treated populations mean root length of the seedling ranged from 3.15 cm (100Gy) to 4.44 cm (350 Gy) over the control (2.87 cm).Highest root length in Gamma rays treated seeds observed at 350Gy and Lowest at 100Gy.

Effect of mutagens on M_1 generation plants under field conditions

The EMS and Gamma radiation treated seeds were sown in the experimental field site in RBD (Randomized Block Design). The important observations were noted viz., Morphological leaf variations, terminal leaf shape, which is shown in the form of figures (Fig.2).

Conclusion:

On the basis of the M_1 generation experiment,decreased seedling height and increased seedling injury show that mutagens used were important in inducing mutations in M_1 plants of *Vigna radiata* (L) Wilczek. the study on mungbean seedling height and seedling injury concluded that environmental stressors like water stress and shading significantly reduce seedling height, potentially leading to lower biomass and yield. Injury is often a consequence of such stress, which impairs physiological functions, alters growth, and can increase disease susceptibility, thereby impacting overall plant health and productivity. Maximum effect of mutagenesis was observed at the 450Gy dose, where the seedling injury was 30.61 %- and 40-mM concentration 33.35 % which was maximum among others. Both mutagenic treatments were best suited to study Seedling height and injury in *Vigna radiata* (L) Wilczek.

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