# Induced Mutagenesis Expands Genetic Base and Drives Cultivar Development in Black Gram

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***Abstract—*** *Black gram is a nutritionally rich grain legume constrained by a narrow genetic base. Induced mutagenesis is a valuable technique for creating novel variability. This study investigated the potential of gamma rays and electron beam irradiation to induce mutations in the black gram variety TU94-2 and characterized the biochemical basis of Urdbean Leaf Crinkle Virus (ULCV) resistance. Seeds were treated with 200, 300, 400, and 500 Gy of each mutagen. The $M_1$ generation was assessed for biological damage, while chlorophyll and morphological mutants were screened in the $M_2$ generation. Mutagenic effectiveness and efficiency were calculated. Selected mutants and control cultivars were evaluated for ULCV resistance and seed sugar composition. The results indicated that 400 Gy gamma rays and 300 Gy electron beams were optimal, causing low plant damage while inducing high genetic effects. Biochemical profiling revealed that the resistant cultivar VBN (Bg) 6 maintained chlorophyll and protein homeostasis under infection, unlike the susceptible CO 5. The study demonstrates that induced mutagenesis is a potent tool for generating valuable genetic diversity for black gram improvement*.

***Keywords— Vigna mungo; Black gram; Mutation breeding; Gamma rays; Electron beam irradiation; Urdbean Leaf Crinkle Virus (ULCV); Disease resistance.***

## I. INTRODUCTION

Black gram (*Vigna mungo* (L.) Hepper), widely recognized as urdbean, is a nutritionally rich grain legume containing approximately 25–28% protein, primarily cultivated in the Indian subcontinent. Despite its value, genetic improvement has been constrained by a narrow genetic base due to its self-pollinating nature (Gupta et al., 2005). Since genetic variation is essential for crop improvement, induced mutagenesis has become a valuable tool for generating new genetic diversity.

Various physical mutagens, including gamma rays, X-rays, and neutrons, have been effectively employed to generate mutants. Over the last eight decades, physical mutagens—especially ionizing radiations such as gamma-rays, X-rays, and neutrons—have been the most extensively used, accounting for over 70% of mutant varieties developed through physical mutagenesis (Mba, 2025). According to the FAO/IAEA Mutant Variety Database (MVD), more than 3,281 officially released mutant varieties with improved traits have been documented. The majority were developed using ionizing radiation, primarily gamma-rays (64%) and X-rays (22%). Since mutation is a random process, the successful recovery of desirable mutants depends on mutagenic efficiency, the genetic quality of the original material, and the effectiveness of the screening procedure (Hase et al., 2025).

In black gram, mutation breeding research began in the 1960s. Research efforts at the Bhabha Atomic Research Centre (BARC) have successfully integrated mutation and recombination breeding to develop several improved cultivars, such as TAU-1, which has seen widespread adoption in Maharashtra. In recent years, besides conventional radiations, electron beam radiation has emerged as a promising alternative. The present study investigates: (1) the potential of electron beam irradiation to induce chlorophyll and morphological mutations in black gram in comparison with gamma-rays, and (2) the biochemical response of contrasting genotypes to Urdbean Leaf Crinkle Virus infection.

## II. MATERIALS AND METHODS

### 2.1 Development of Mutant Population:

Genetically pure, uniform, and dry seeds (12% moisture content) of the black gram variety TU94-2 were subjected to mutagenic treatments. For gamma irradiation, seeds were exposed to four doses (200, 300, 400, and 500 Gy) of ^60Co gamma-rays at the Bhabha Atomic Research Centre (BARC), Trombay, India. For electron beam irradiation, seeds were treated with 7.5 MeV electron beams at the same doses at the Electron Accelerator Facility, Raja Ramanna Centre for Advanced Technology (RRCAT), Indore, India. Aluminium scatterers were employed to achieve a dose rate of ~8 Gy/s.

**FIGURE 1: Seeds of black gram (*Vigna mungo* (L.) Hepper) variety TU94-2 used as the base material for mutagenic treatments.**

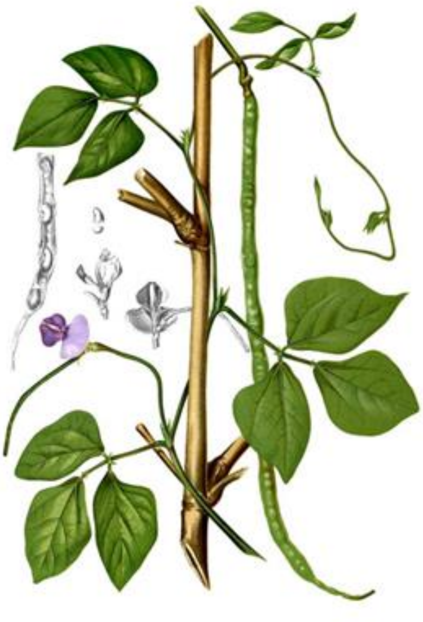

**Figure 2. Botanical morphology of black gram (*Vigna mungo* (L.) Hepper) showing vegetative and reproductive structures**

Treated and untreated control seeds were sown at the experimental field facility, BARC, to raise the $M_1$ generation. Observations on seedling injury and lethality were recorded. $M_1$ plants were harvested separately to establish plant progeny rows in the $M_2$ generation. In the $M_2$ generation, chlorophyll-deficient and viable morphological mutations were recorded from emergence to physiological maturity. Data on biological damage in $M_1$ and mutation frequency in $M_2$ were used to estimate mutagenic effectiveness and efficiency, following Konzak et al. (1965).

Desirable mutants were selected in $M_2$, tagged, and harvested individually to raise the $M_3$ generation. Selection in $M_3$ focused on identifying homozygous lines exhibiting desirable traits. True-breeding mutants were advanced for stability confirmation.

### 2.2 Screening for Urdbean Leaf Crinkle Virus (ULCV) Resistance:

The $M_2$ population was examined in a Trombay ULCV hotspot area. Each plot consisted of a 3 m row with a 30 cm gap between rows. Ten plants per meter were scored for disease symptoms based on leaf crinkling and chlorosis. Disease reaction was scored on a 1-5 scale (Table 1) based on the percentage of infected leaves or plants per row.

**TABLE 1**
**DISEASE RATING SCALE FOR URDBEAN LEAF CRINKLE VIRUS**

| S. No. | Category | Percentage of infection | Disease rating score |
|---|---|---|---|
| 1 | Resistant | 0–10% | 1 |
| 2 | Moderate resistant | 11–30% | 2 |
| 3 | Moderate susceptible | 31–40% | 3 |
| 4 | Susceptible | 41–75% | 4 |
| 5 | Highly susceptible | 76–100% | 5 |

For ULCV, resistance was scored based on the absence of leaf crinkling and chlorosis. Every fifth row, the highly susceptible cultivar LBG17 served as an infector row. Customary cultural practices were observed, except for the absence of insecticide spraying.

### 2.3 Biochemical and Sugar Composition Analysis:

To characterize the physiological basis of resistance, the biochemical response of a known resistant cultivar, VBN (Bg) 6, and a susceptible cultivar, CO 5, to ULCV infection was analyzed. Chlorophyll, total sugar, soluble protein, and phenol content were measured in mock-inoculated and virus-inoculated plants.

For sugar composition analysis of mutant seeds, mature seeds ($M_3$) were steeped in distilled water at room temperature overnight. Approximately ten seeds were ground in ten millilitres of 80% ethanol. The slurry was shaken for five hours, then centrifuged for 20 minutes at 12,000 rpm (20,913 ×g). The clear supernatant was dried in a vacuum concentrator at 45°C. The dried residues were suspended in 75 µl of acetonitrile:water (65:35 v/v), centrifuged, and 4 µl of supernatant was injected for HPLC analysis.

Analysis was performed using a JASCO HPLC System equipped with a carbohydrate column (30 cm × 3.9 mm) and acetonitrile:water (65:35 v/v) as the mobile phase at a flow rate of 1.0 mL/min. Sugars were identified by comparison with authentic standards. Concentrations were quantified using standard calibration curves and expressed as milligrams per gram of seed meal. Five replications per sample were analyzed.

## III. RESULTS

### 3.1 Mutagenic Effectiveness and Efficiency:

Among the different doses tested, 400 Gy gamma rays and 300 Gy electron beams proved to be the most effective, causing minimal plant damage while producing strong genetic effects. Data on biological damage (injury and lethality) in the $M_1$ generation and mutation frequency in the $M_2$ generation were used to estimate mutagenic effectiveness and efficiency.

**FIGURE 3: Effect of increasing electron beam irradiation exposure on early seedling growth of black gram.**


**FIGURE 4: Effect of electron beam irradiation exposure duration on early growth, biomass accumulation, and chlorophyll content of black gram seedlings**

### 3.2 Chlorophyll and Morphological Mutations:

Chlorophyll mutations are one of the most reliable markers for evaluating the genetic impact of mutagenic treatments. In this investigation, compared to gamma-rays, more viable chlorophyll mutations and lethal mutants were found at greater doses of electron beam irradiation. Among the various types of chlorophyll mutations, *xantha* was the most common (2%–4%), followed by *chlorina* and *albino* in both gamma-ray and electron beam treated populations. A similar pattern was noted in chickpeas.

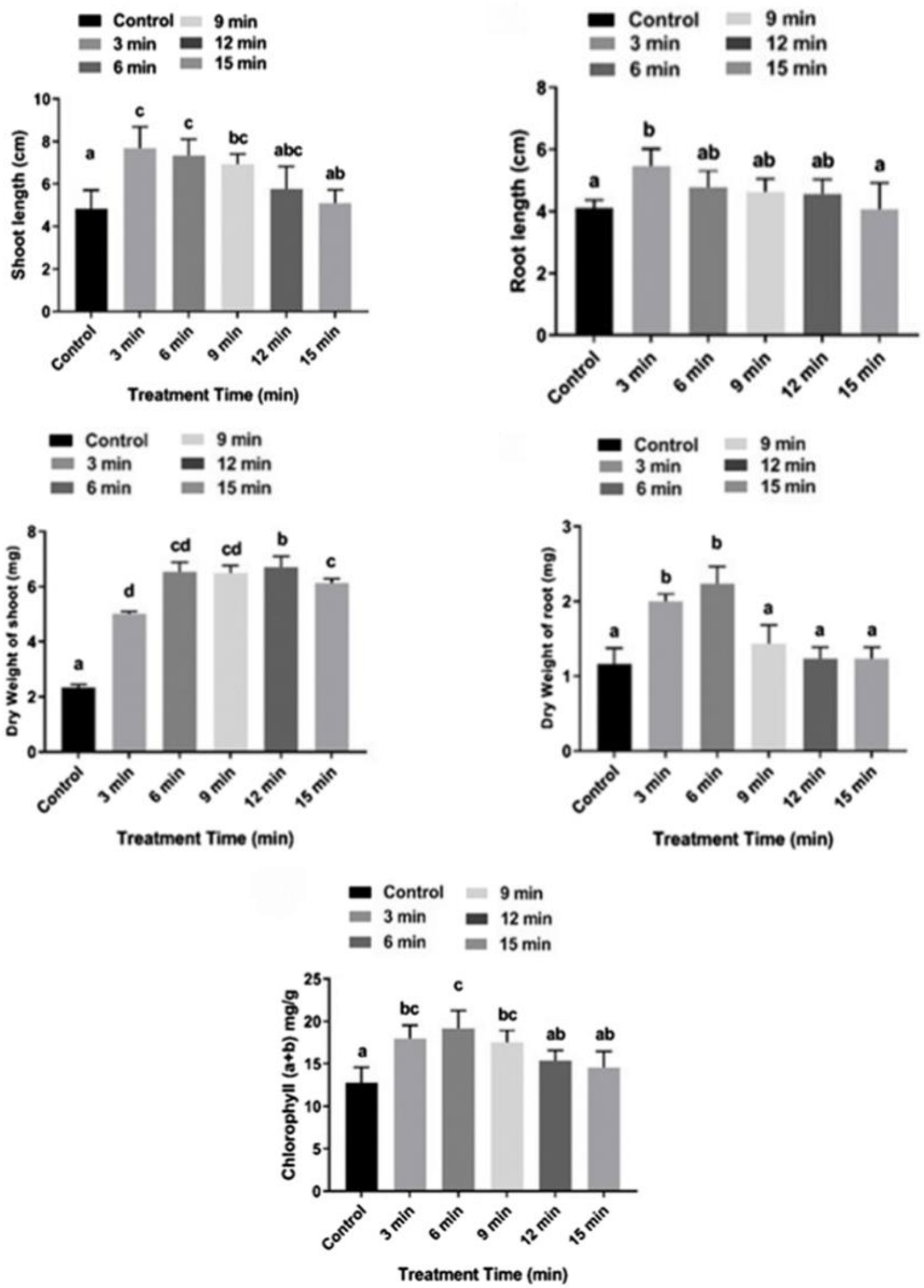


**FIGURE 5: Effect of electron beam irradiation exposure duration on early growth and physiological traits of black gram (*Vigna mungo* (L.) Hepper) seedlings. Bars represent mean ± SE for (a) shoot length, (b) root length, (c) dry weight of shoot, (d) dry weight of root, and (e) total chlorophyll content (a + b). Different letters above bars indicate significant differences at $p < 0.05$.**

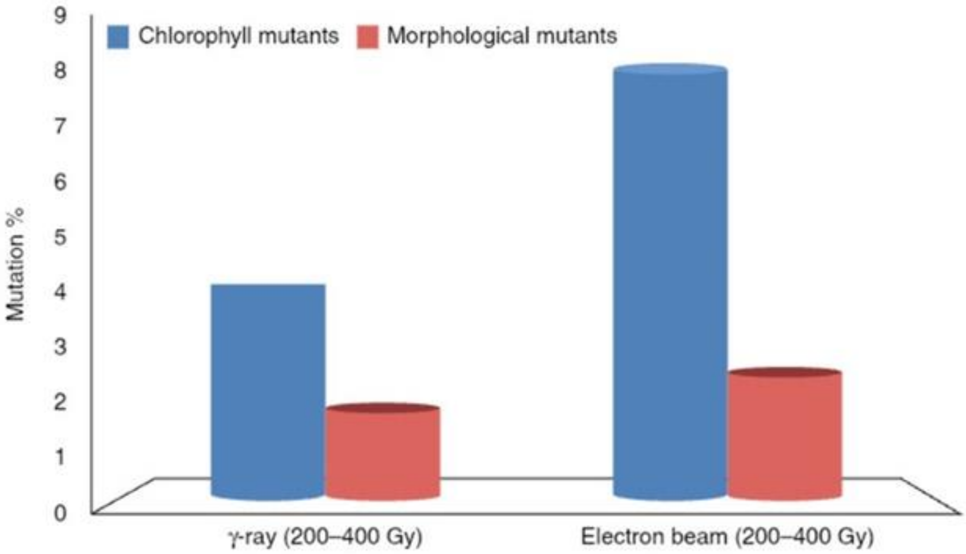


**FIGURE 6: Chlorophyll and Morphological Mutations**

### 3.3 Genetic Variability and Selected Traits:

The study revealed a considerable level of genetic variability among irradiation-derived mutants when compared to their original parent plants. Desirable mutants were selected in the $M_2$ generation. In the $M_3$ generation, selection was focused on identifying homozygous lines exhibiting desirable traits. High-yielding and synchronously maturing plants were identified and bulked.


**FIGURE 7: Spectrum of phenotypic variability induced through mutagenesis in black gram (*Vigna mungo* (L.) Hepper). Panels (a–n) show representative morphological mutants exhibiting variation in leaf shape, size, and plant architecture, while panels (o–ab) depict seed morphology mutants differing in size, shape, and surface characteristics in the advanced mutant generations.**

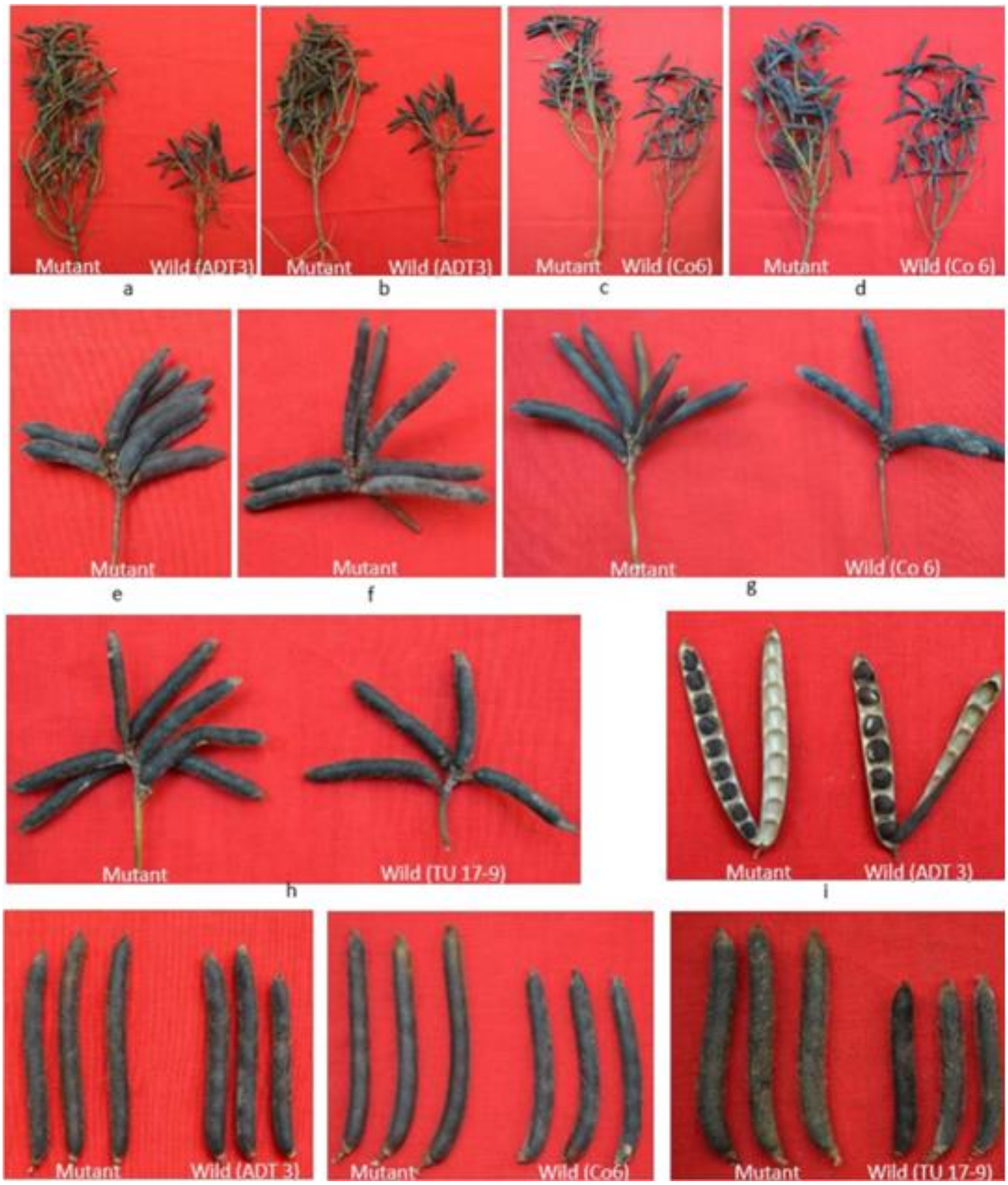


**FIGURE 8: Comparative pod morphology of selected black gram (*Vigna mungo* (L.) Hepper) mutants and their respective wild-type cultivars. Panels show differences in pod number per cluster, pod length, and seed filling between mutant lines and controls (ADT 3, CO 6, and TU 17-9).**

### 3.4 Biochemical Response to Urdbean Leaf Crinkle Virus Infection:

The differential biochemical response of a resistant (VBN (Bg) 6) and a susceptible (CO 5) black gram cultivar to ULCV infection was analyzed (Table 2). Infection caused a severe decline in photosynthetic pigments (chlorophyll a, b, and total chlorophyll) in the susceptible cultivar CO 5, with reductions exceeding 30%. In contrast, the resistant line VBN (Bg) 6 maintained stable chlorophyll levels, showing reductions of less than 9%.

Virus infection also significantly altered primary and secondary metabolism. The susceptible CO 5 exhibited a large increase in total sugar content (+37.93%) coupled with a sharp decrease in total soluble protein (-26.96%). The resistant VBN (Bg) 6, however, showed only moderate increases in total sugar (+6.86%) and total soluble protein (+7.07%). Furthermore, while total phenolic content decreased in the susceptible cultivar, it increased in the resistant one.

**TABLE 2**
**DIFFERENTIAL BIOCHEMICAL RESPONSE OF VBN (Bg) 6 AND CO 5 TO URDBEAN LEAF CRINKLE VIRUS INFECTION**

| Trait | Cultivars | Mock inoculated (mg/g FW) | Inoculated (mg/g FW) | % of change |
|---|---|---|---|---|
| Chlorophyll a | VBN (Bg) 6 | 1.15 | 1.07 | −6.96 |
| | CO 5 | 1.01 | 0.67* | −33.66 |
| Chlorophyll b | VBN (Bg) 6 | 0.91 | 0.83 | −8.79 |
| | CO 5 | 0.84 | 0.58* | −30.95 |
| Total chlorophyll | VBN (Bg) 6 | 2.06 | 1.9 | −7.77 |
| | CO 5 | 1.85 | 1.25* | −32.43 |
| Total sugar | VBN (Bg) 6 | 5.25 | 5.61 | 6.86 |
| | CO 5 | 5.8 | 8.00* | 37.93 |
| Total soluble protein | VBN (Bg) 6 | 5.52 | 5.91 | 7.07 |
| | CO 5 | 6.12 | 4.47* | −26.96 |
| Total phenols | VBN (Bg) 6 | 4.05 | 4.32 | 6.67 |
| | CO 5 | 3.1 | 2.81 | −9.35 |

***Values marked with an asterisk () indicate a statistically significant difference from the mock-inoculated control (p < 0.05).****

## IV. DISCUSSION

The present study confirms that induced mutagenesis using gamma rays and electron beams is a powerful technique for generating novel genetic variability in black gram. The optimal doses of 400 Gy gamma rays and 300 Gy electron beams, which produced high genetic effects with low plant damage, align with the concept that an ideal mutagenic treatment balances mutation induction with acceptable biological damage. Chlorophyll mutations, governed by approximately 250–300 loci, served as a sensitive indicator of genomic disruption, with *xantha* types being most frequent, consistent with reports in other *Vigna* species.

The differential biochemical response to ULCV infection provides insights into the physiological basis of resistance. The resistant genotype VBN (Bg) 6 demonstrated a clear ability to maintain biochemical homeostasis under viral stress, preserving photosynthetic machinery and protein synthesis while mounting a moderate defense-related phenolic response. In contrast, the susceptible CO 5 suffered severe chlorophyll degradation, a disruptive surge in soluble sugars (likely due to blocked translocation), and a collapse in protein content. This pattern suggests that resistance is associated with the maintenance of source-sink dynamics and cellular integrity during infection.

The mutants identified in this study, particularly those with improved agronomic traits, can serve as valuable sources of variation. Those exhibiting biochemical profiles analogous to the resistant check VBN (Bg) 6 are prime candidates for further development in virus-resistance breeding programs. Sustained enhancement of plant productivity is essential to meet increasing global food demand and climate challenges. Induced mutations will continue to play a vital role in developing improved crop varieties with traits such as better protein quality, enhanced nutrient uptake, and greater tolerance to biotic and abiotic stresses.

## V. CONCLUSION

This research successfully demonstrates the efficacy of gamma ray and electron beam irradiation in creating useful genetic variability in black gram. The identified optimal doses provide a guideline for future mutation breeding work in this crop. Furthermore, the biochemical characterization of virus resistance establishes key physiological markers—chlorophyll retention, stable protein levels, and controlled sugar accumulation—that can be used for screening mutant populations. The developed mutant lines offer valuable genetic resources for direct cultivation or for use as parental lines in hybridization programs aimed at developing high-yielding, virus-resistant black gram varieties. This integrated approach of mutation induction and physiological screening contributes significantly to strategies for sustainable pulse production.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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