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## Research Article

# Seed Application of Gamma Radiation in Guar (*Cyamopsis tetragonoloba*): Boosting Yield and Agronomic Traits through Induced Mutagenesis

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## ABSTRACT

Guar (*Cyamopsis tetragonoloba*) is a drought tolerant legume crop, plays an important role in agriculture and has versatile uses in different industries. The objective of study was to investigate the effects of gamma radiation on the yield characters of the guar variety "BR-21" because conventional breeding approaches are limited in guar yield improvement due to narrow genetic diversity. For this purpose, seeds of guar variety were treated with different doses (10 to 50 kR) of gamma radiation and agronomic parameters were analyzed in field experiment. From the results it was cleared that low to moderate doses (10–20 kR) increase the yield characters such as clusters per plant, pod length, and grains per pod. Particularly, 20 kR dose proved to be the most efficient dose, greatly enhanced growth and reproductive characters without any adverse effects. Higher doses (30–40 kR), on other hand resulted physiological and genetic damage, growth inhibition, chlorosis, and yield decline. These findings conclude that 20 kR is an effective dose to increase guar yield by creating positive genetic mutations without any adverse effects. The findings from this study provide new insights for mutation breeding programs to enhance crop productivity and also fulfill the demand of guar gum.

**Keywords:** BR-21 variety, Diversity, Guar, Gamma radiation, Grain yield, Mutagenesis, Plant growth

## INTRODUCTION

Guar (*Cyamopsis tetragonoloba*), a drought-tolerant legume crop and plays an important role in sustainable agriculture because of its ability to fix atmospheric nitrogen by symbiotic relationship with rhizobia, enhancing soil fertility and reducing dependence on synthetic fertilizers (El-Sawah *et al.*, 2021). Its industrial importance increased by the surging demand of guar gum- polysaccharides compound with thickening, stabilizing, and emulsifying characteristics which are intensively used in food, pharmaceuticals, and cosmetics and petroleum industries (Khurizadeh *et al.*, 2024; Sarri *et al.*, 2024). Guar also has medicinal importance rather than industrial utilization, to reduce constipation, indigestion problems, anemia due to its galactagogue, digestive, and tonic properties (Sharma *et al.*, 2011). Moreover, its importance further enhanced because of high soluble fiber content that is helpful in controlling cholesterol level (Pande *et al.*, 2012). Despite these advantages global guar yield remains suboptimal due to some genetic, biotic and abiotic factors, i.e., narrow genetic variability, high self-pollination rate reduced diversity improvement



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through conventional breeding is difficult due to minute flower size, and recent cultivars produce optimum yield and do not have significant potential because of its narrow genetic variability (Kumar *et al.*, 2017; Abdulhafiz *et al.*, 2018). On the other hand, drought considering another factor that affect the guar yield globally (Khanna-Chopra and Singh, 2015), generally guar is considered as drought and heat tolerant crop (Undersander *et al.*, 2023; Sultan *et al.*, 2013) but according to recent studies it is mentioned that guar genotypes exhibiting diversity in response to different irrigation levels and guar yield decreases with drought stress at different growth stages (Ali *et al.*, 2015; Zubair *et al.*, 2017; Avola *et al.*, 2020). So, to overcome these problems, scientists have investigated induced mutagenesis, mainly focusing on gamma radiation, as an easy and inexpensive way to create genetic variability and increase necessary agronomic characters such as plant height, increase pod formation, and finally seed yield (Khurizadeh *et al.*, 2024; Sarri *et al.*, 2024).

Researchers have been efficiently applied gamma radiation on other crops to incorporate genetic mutations and improve many characters without changing their original genetic pattern. Earlier research has confirmed its potential to increase biotic and abiotic stress resistance, enhance growth, and increase genetic variability (Khaled *et al.*, 2022; Riviello-Flores *et al.*, 2022). However, the work of many researchers has focused on stress resistance, and general crop development but research on optimizing radiation doses specific for yield-related traits in guar is limited.

This research gap still exists to identify the optimum radiation dose specific to increase guar's yield. To keep in view this gap, a research plan requires to investigate which specific radiation dose can increase yield characters such as seed yield, pod length, pods per plant, pods per cluster, and plant height. This study made a hypothesis that low to medium gamma radiation doses can induce valuable mutations, resulting in enhanced guar's yield and adaptability.

The main objective of this study was to detect the most beneficial gamma radiation dose/s for increasing yield traits in guar, to evaluate the long-term performance and stability in productivity of irradiated plants of guar and to find out relationship of specific radiation dose/s to improve of yield characters. By focusing on the above objectives, this research aims to increase guar cultivation with high productivity and to fulfill the global demand of guar gum. The outcome of study will also provide actionable facts, linking the gap between basic research and its real applications, so contributed to economy and fulfill industrial demand.

## MATERIALS AND METHODS

### Plant material and seed source

Guar (*Cyamopsis tetragonoloba*), mainly cultivated in tropical and subtropical regions. Seeds of the "BR-21" variety were procured from the Agricultural Research Station (ARS), Bahawalpur. Agronomic and morphological evaluations of the plants were carried out under the supervision of a plant breeding specialist.

### Gamma radiation of seeds

The seeds of approved guar variety "BR-21" were prepared for gamma radiation treatments. For each radiation dose, 50 g of dry seeds were packed in sealed zip-lock bags. Gamma radiation was administered using Cs-137 radiation sources at dose levels of 10 kR, 20 kR, 30 kR, 40 kR, and 50 kR, along with a non-irradiated (0 kR) as a control. The radiation process was performed at the Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan during year 2021. The un-treated seed used as baseline to check the performance of other doses of gamma radiation.

### Experimental site and growth conditions

The radiated seeds including control treatment were sown at the experimental field of Agricultural Research Station, Bahawalpur during June 2022. The sowing was completed with hand drill in randomized complete block design (RCBD) including three replications and all treatments were assorted randomly. The randomization process was started before sowing during layout as the five treatments were arranged in ascending order (0 kR to 50 kR) and allotted them numerical numbers (1-6). The random number generator website used to minimize human preference; computers generate a list randomly. Write the treatment as list generated by computer. The plot size kept 17 feet x 9 feet and 45 cm row to row distance were maintained and each treatment was sown in 6 rows, while all agronomic and cultural practices were conducted during the experiment.

### Study design

The experiment was designed to evaluate the effects of gamma radiation as a control untreated seed) and a treated seed (five gamma radiation doses ranging from 10–50 kR). The rationale for selection of radiation doses because low doses radiation (10-30 kR) are sub-lethal and more effective and induced beneficial mutations with minimum affecting the plant growth and function, while higher doses (40-50 kR) induce more drastic affect often associated with physiological, biochemical changes that mostly reduced the growth and yield, but the higher doses are essential to determine threshold levels of mutagenic stress (Soliman & Amin, 2025; Abozahra *et al.*, 2025)

## Morphological parameters

The morphological traits of the plants were assessed at maturity by recording data and through image analysis by ChatGPT (Murphy *et. al.*, 2024) to determine the impact of gamma radiation on plant growth and physiology. The following parameters were recorded (plant height, pods per plant, pods per cluster, pod length, clusters per plant, grains per pod, general plant health). Each parameter was measured across three replications, and the averaged data were analyzed to determine the radiation effects.

## Statistical Analysis

Agronomic data were statistically analyzed ANOVA and post-hoc test (HSD) to check the significance level of gamma radiation treatments on morphological characters. The Statistics 8.1 software used to conduct the analysis.

## RESULTS

### Analysis of variance (ANOVA) and effect of different radiation treatments on plant parameters

Table 1 shows an analysis of variance (ANOVA) of different morphological traits of guar variety "BR-21" treated with different doses of gamma radiation. Mean square (MS) = (Plant height= 399; clusters per plant= 8.2; pod length= 3.7; pods per plant= 5195; pods per cluster= 8.2; grains per pod= 18.4) indicated that the treatment application with different doses of gamma radiation has significantly influenced the all-agronomic traits of guar except plant height and conclude that treatment variance is higher than error variance, showing that the variation in measured characters are due to radiation treatments, rather than random variation.

Table 1. ANOVA (Mean Squares) of morphological traits of guar (*C. tetragonoloba*) variety "BR-21"

SOV	DF	Plant Height	Clusters per plant	Pod length	Pods per plant	Pods per cluster	Grains per pod
Treatment	4	399n.s	8.2**	3.7**	5195**	8.2**	18.4**
Error	8	255	0.4	0.5	805	0.4	2.7

SOV: source of variation, DF: degree of freedom, n.s: non-significant; \*\*: highly significant at  $P > 0.005\%$ .

Table 2. HSD test based mean comparison of morphological traits of guar (*C. tetragonoloba*) variety "BR-21"

Treatment	PH (cm)	CPP	PL (cm)	PPP	PPC	GPP
0 kR)	108.23A	19.2A	5.6AB	117.8A	6.2A	8.6A
10 kR	106.4A	12.4B	6.4A	70.2AB	5.6A	9.0A
20 kR	114.2A	20A	4.6B	119A	6.0A	6.8AB
30 kR	116.8A	16.6AB	4.0B	58.6B	3.6B	4.6AB
40 kR	105A	6.6C	3.6C	15.6C	2.4B	3.8B

Note: Treatments not sharing the same letter, significantly different ( $P > 0.005\%$ ). CPP: Clusters per plant; PL: Pod length; PPP: Pods per plant; PPC: Pods per cluster; GPP: Grains per pod






The HSD test conducted to compare mean values of agronomic traits across different gamma radiation doses. The 0, 10 and 20 kR treatments are statistically similar in same group (A), while 30, 40 kR are another group (B) significantly reduced clusters per plant that higher doses have negative impact on grain yield. The pod length has positive impact at low dose and showed highest pod length (6.3 cm) that is statistically superior to 30 & 40 kR, while 0 and 20 kR showed intermediate response to pod length. So, mild doses enhance the pod elongation, but higher doses reduce them. The only one treatment 40 kR showed significant negative impact to reduce the pods per plant, while other treatments showed tolerance of radiation stress.

The 0, 10, 20 kR treatments exhibited a stable performance for pods per cluster, while 30, 40 kR significantly reduced the pod setting per cluster, while as concerned with grains per pod the lower doses 0, 10 kR were best and 20 kR intermediate but 30, 40 kR reduced the grains per pod (Table 2). While the 50 kR dose is a lethal dose and no germination of plants at higher dose.

## AI-based image analysis

The AI-based image analysis of BR-21 guar plants under varying gamma radiation doses reveals a clear dose-dependent impact on growth, physiology, and yield (Table 3). At 0 kR (control), plants showed natural, vigorous growth with no stress signs, while a low dose of 10 kR caused mild reductions in height and early physiological changes, but still managed marginal yield improvement. Interestingly, 20 kR emerged as the most beneficial treatment—plants showed optimal growth, enhanced branching, improved photosynthetic efficiency, and the highest yield due to better pod and grain production.

Table 3: AI-based image analysis of guar plants of BR-21 variety on growth, physiological and yield parameters.

Dose	0 Kr	10 kR	20 kR	30 kR	40 kR
Plant Growth characters	Vigorous growth, dense canopy, natural traits	Mild reductions in height and branching	Optimal growth, enhanced branching	Stunted growth, reduced leaf size	Severe growth inhibition, chlorosis
Physiological parameters	Optimal photosynthesis, no stress	Slight chlorophyll decline, early mutations	Improved photosynthesis, stress adaptation	Metabolic disruption, oxidative stress	Poor photosynthesis, cellular damage
Yield	Baseline performance	Marginal yield stimulation	Increased pod and grain production	Significant yield decline	Minimal yield, poor adaptability
Phenotype					

However, at 30 kR, plants began to show clear stress symptoms, including stunted growth and metabolic disruption, leading to a sharp decline in yield. The highest dose, 40 kR, proved toxic, causing severe growth inhibition, chlorosis, poor photosynthesis, and almost negligible yield. These observations suggest that while moderate radiation can stimulate beneficial mutations and adaptive responses, excessive exposure disrupts cellular function and significantly hampers productivity.

## DISCUSSION

Gamma radiation is mostly utilized to create induced genetic variability and used as a powerful tool in mutation breeding programs to improve crops potential (Jain, 2010; Hong *et al.*, 2014). In this experiment, different gamma radiation doses were applied to guar variety BR-21 to evaluate the mutation effects on growth and yield related traits. The outcome of this study determines how various doses act as stimulators or stressors, and how specific doses can help achieve breeding objectives. Bahawalpur is the only agricultural research station that has conducted research on guar and guar varieties already adopted and selected in high temperature conditions. It is also clear from literature that guar tolerate the drought conditions with high temperature. Based on the above discussion, it is concluded that soil and environmental conditions have a limited or no chances to affect the results (Pandey *et al.*, 2022; Vishnyakova *et al.*, 2023; Kumari & Prasad, 2024). The environmental factors like high temperature at early growth stages may affect the results of experiment and this experiment conducted in Bahawalpur location which already in high temperature range and Agricultural Research Station, Bahawalpur is only station that conducted research on guar, so the guar varieties already adopted and selected in local high temperature conditions and from literature it is also cleared that guar has drought tolerant crop and can survive on high temperature, it is concluded from above discussion that soil and environmental conditions have limited affected the results of experiments.

### Baseline performance of untreated BR-21 and mild stimulation at low doses (10 kR)

The untreated control group (0 kR) showed vigorous growth, dark green leaves, and upright branching, indicating stable photosynthesis and genetic integrity. The superior performance in yield contributing characters further confirmed the natural potential of BR-21. This is consistent with earlier reports (Ahloowalia & Maluszynski, 2001).

The effects of low dose (10 kR) bring a mild stimulation with positive changes in BR-21. There is a slight reduction in morphological parameters, including plant height, slight thin leaves, and reduced in branching. This likely cause the activation of stress-related pathways, increased in proline and soluble sugar, minor changes in hormonal balance (slightly increase in auxins and cytokinins), and DNA changes at low level inducing beneficial mutations (Kiong *et al.*, 2008; Shahwar *et al.*, 2021). It has been also noted that no doubt there is slight reduction in the above morphological

parameters but plants also showed highest number of grains per pod, and pod length at this dose as compared to the control, so indicating the stress activation in seed development with cell elongation structures. While 10 kR also represents the genetic potential to improve specific yield characters, such as the highest number of pod grains and increase in plant clustering overall enhance the grain production. Such low-dose-induced hormesis and enhanced seed development have also been noted recently in lentil and soybean (Yasmeen *et al.*, 2022; Bashir *et al.*, 2023).

#### **Optimal enhancements at moderate doses (20 kR)**

The 20 kR dose appeared to be an optimal dose to enhance vegetative and reproductive traits. The plants showed improved chlorophyll content, branching and reproductive structures. The 20 kR dose upregulated antioxidants enzymes (SOD, CAT, POD), improve nutrients assimilates and activate genes linked to enhance photosynthetic efficiency and reproductive structures (Naik *et al.*, 2009; Shikazono *et al.*, 2021). Recent research showed that moderate gamma doses can beneficially modulate gene expression related to stress response, metabolic homeostasis, and yield (Al-Safadi *et al.*, 2021; Mishra *et al.*, 2023).

The plants of guar variety "BR-21" treated with 20 kR dose exhibited the excellence performance in morphological parameters, including plant height, healthy and vigorous leaves, with improve branching pattern, proving an ideal dose for positive stress and stimulation in guar variety "BR-21". This dose stimulates and enhances photosynthetic activity, resulting in an increase in chlorophyll content and efficient nutrient uptake, providing greater food and energy to complete reproductive structures. To support the above statement evidence includes the highest number of clusters and pods per plant ultimately increase the grain yield. The significant improvements were observed at 20 kR as compared to 10 kR due to moderate stress which stimulates and enhance growth and reproductive activities to improve the yield traits. The findings of positive stress by low to moderate doses also mentioned by other researchers in their studies (Calabrese & Baldwin, 2001; Mohajer *et al.*, 2014).

#### **Adverse Effects on plants at high doses (30 kR, 40 kR)**

It has been also noted that as doses exceed 20 kR adverse effects were observed because plants treated with 30 kR, 40 kR showed stunted growth with thin and few leaves and even reduced to no clusters. These doses likely to increase ROS accumulation, overwhelming the antioxidant system and causing lipid peroxidation, protein denaturation, and DNA fragmentation (Jan *et al.*, 2012; Kovács & Keresztes, 2002). Chromosomal aberrations, mitotic spindle disruptions, and hormonal imbalances (elevated ABA, suppressed gibberellins) could explain reproductive failure and poor seed set. Recent work supports these findings, showing that high gamma doses cause DNA double-strand breaks, impaired gametogenesis, and reduced seed viability in various crops (Bhattacharya *et al.*, 2022; Shafique *et al.*, 2021; Chaurasia *et al.*, 2024).

These changes were due to disruption in metabolic activities by the cause of oxidative stress and chromosomal damage. At 40 kR, extreme chlorosis with tender weak stems, and yellow thin leaves. The significant decline in the number of pods per cluster and grains per pod indicates an unrepaired reproductive mechanism. These doses interrupted the important functions such as photosynthesis activities, hormonal imbalance, and uncomplete cellular structures, also confirming from the earlier studies that identify adverse effects of high doses of gamma radiation (Kiong *et al.*, 2008).

## **CONCLUSION**

This study successfully identifies the most effective dose (20 kR gamma radiation) to enhance yield-related characters in guar (*Cyamopsis tetragonoloba*) variety "BR-21". The findings elaborate that moderate radiation dose trigger the positive hormonal effects and produce beneficial physiological and genetic changes. This optimal dose brings positive change and maintains the balance between beneficial genetic mutations and plant health, finally achieving primary objective of study. While the other doses like 10 kR, strike mild stimulation with slight improvements in few characters such as pod length and number of grains but overall improvement in yield-related characters were less as compared to 20 kR. On the other hand, higher doses (30 kR, 40 kR) produced adverse effects, and yield declines in guar variety. This study is conducted in the soil and environmental conditions of Bahawalpur; the results are limited to local environment. The results may be changed in other environmental and soil conditions.

## **AUTHOR CONTRIBUTIONS**

All authors contributed substantial and direct contribution to this research and approved it for publication.

## **CONFLICT OF INTEREST,**

The authors declare that they have no conflict of interest.

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