

## Research Article



## Effects of Gamma Irradiation on some Growth Attributes in Cotton (*Gossypium hirsutum* L.)

Saleem Khan<sup>1</sup>, Amir Hamza<sup>2</sup>, Farhadullah Khan<sup>3</sup>, M. Subhan<sup>4</sup>, Aziz Khan<sup>1</sup>, Irfan Ali Shah<sup>1</sup>, Shakirullah Khan Shakir<sup>3\*</sup>

<sup>1</sup>University of Science and Technology Bannu, KPK; <sup>2</sup>Qaid e Azam University, Islamabad; <sup>3</sup>Hazara University, Mansehra, KPK; <sup>4</sup>Shabeed Benazir Bhutto University, Sheringal, Dir Upper, KPK; <sup>5</sup>University of Science and technology, KUST, Kohat, 26000, Pakistan.

**Abstract** | For the analysis of gamma rays-induced changes in different growth related end point using cotton as a model plants, the present study was designed. For this purpose, dry seeds of four cotton genotypes (Gomal-93, Bt-131, Bt-121 and Bt-CIM-602) were exposed to gamma rays with 10, 15, 20 and 25 Kilo Radium (KR) doses sourced from <sup>60</sup>Co. The irradiated seed samples were assumed treated seeds while non-irradiated seeds of each genotype were used as control. Field experiment was conducted in randomized complete block design (RCBD) in three replicates in the green house. Radiation effects were observed on some agronomic traits like Plant height, Number of branches plant<sup>-1</sup>, number of bolls plant<sup>-1</sup>, Fiber Fineness and yield of seed. The analysis of variance (ANOVA) revealed significant differences for radiation treatments, genotypes and interaction for treatments × genotypes regarding plant height, number of branches plant<sup>-1</sup>, number of bolls plant<sup>-1</sup> and yield of cotton seeds plant<sup>-1</sup>. Higher levels of gamma rays (20 and 25 KR) effected most of the parameters adversely. Significant reduction in plant height was observed for all the genotypes under the influence of the gamma rays treatments. Increased level of radiation posed adverse effect on number of branches plant<sup>-1</sup>. However, in case of Bt -121, branches were enhanced in treated as compared to control. Similarly, rise in the number of bolls in the seedlings of Gomal-93, Bt-131 and Bt -121 were observed which seeds were exposed to 20 KR dose of radiation. The findings of the present investigations revealed that irradiation of seeds with rays can improve the growth parameters in plants developed the irradiated seeds.

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**\*Correspondence** | Shakirullah Khan Shakir, Hazara University, Mansehra, KPK, Pakistan; **Email:** drshakir0177@gmail.com

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### Introduction

Recently for the better development of crops, the nuclear techniques are widely used in agricultural sectors for improved genetic diversification. As in the conventional techniques, new genetic combinations have been developed from the parental genetic combinations while the nuclear techniques involve the new genetic combinations having frequency of high mutation. Application of nuclear technology involve

the use of ionizing nuclear radiations that inducing mutation in exposed plants and improve the crops quantitatively as well as qualitatively. The induced mutations in the plants exposed to nuclear radiation, might play a promotive role with greater economical values. Literature, survey revealed that mutations induced by Gamma irradiations in the crops have been developed with improved characters having greater potential of increased yield, in time maturity, high protein proportion and healthy stem (Rehman et al.,

1987; Javed et al., 2000). Similarly, Khatri et al. (2005) developed three mutants of *Brassica juncea* L. with greater grain production and early maturation qualities using gamma rays. Shah et al. (2001) successfully developed a variety of oil seed of *Brassica napus* using different ranges of gamma rays and the developed variety was found highly productive, with higher quality of resistant against blight caused by *Alternaria* and the white rust.

Cotton (*Gossypium hirsutum* L.) an allotetraploid systematically belongs to order Malvales, family Malvaceae and genus *Gossypium*. Naturally it is a perennial but often assumed as an annual crop and cultivated in different regions of the world (Smith, 1995). In Pakistan the cotton crop grown in summer from May to mid-June. Cotton is the most important source of natural fiber for the textile industries and also producing edible oil as well as food for castles. Among the cotton producers, Pakistan ranked fifth in the world, by growing on 3 million hectares and cultivated on 15% of the country's agriculture area. Similarly, cotton earning 55% of the foreign exchange and 10% of the total GDP of the country (GoP, 2014). In Pakistan the plain areas especially in Punjab and Sindh produces best quality fiber and can meet the need of textile industry. Cotton assumed to be the best cash crop and plays a pivotal role in the betterment of country's economy by earning the most foreign exchange, as a source of fiber for the textile industries and oil production from the seeds.

To improve the quality and quantity of cotton varieties, yield is the important factor for the selection of cotton varieties which gave profitable production (Bridge et al., 2001). Induced mutation in this regard have now well established technique used for the production of use full mutants (Muller, 1927; Stadler, 1929; Irfaq and Nawab, 2003). In the present investigation there were developed artificial genetic variability in the genotype of cotton local varieties to compare various growth parameters with that of local varieties. The outcomes of the present investigation will be helpful in developing strategies for cotton production, which may best adaptable cotton mutants and can successfully grow in this new locality that ultimately provide a good source of income to the people improving standard of the life via agro based industries in the area.

## Materials and Methods

### Experimental design

Field trials were conducted in green house at the De-

partment of Botany, Faculty of Biological Sciences, University of Science and Technology Bannu (USTB), Khyber Pakhtunkhwa, Pakistan. The germplasm used were consist of four commercially grown varieties of cotton (*Gossypium hirsutum* L.) i.e. V1-(Gomal-93), 2- V2-(Bt-131), V3-(121) and V4-(Bt-CIM-602). Each of the variety was irradiated by exposing to 10, 15, 20 and 25 Kr gamma rays doses from <sup>60</sup>Co source at Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar, Pakistan. Row to row spacing was kept 75 cm and plant to plant distance within the rows was kept 30 cm, in complete block design in a randomized triplicate. All the required agronomical practices and crop protection measures were availed. Each entry of treatment were planted in a 3.3 meter long row keeping ten plants experimental while two plants were left as control, one at each end of the row. The data were recorded for considering different parameters as end points like weight of lint plant<sup>-1</sup>, lint percentage (GOT %) and staple length, staple strength and uniformity index as detailed below.

### Weight of Lint plant<sup>-1</sup>

Weight of lint was also recorded and weighed in grams after ginning the seed cotton of all the treatments.

### Lint Percentage (GOT %)

Clean and dry samples of seed cotton were weighed and then ginned separately with a single roller electric gin. The lint obtained from each sample was weighed and lint percentage was computed by using the following formula.

$$\text{Lint percentage (GOT \%)} = \frac{\text{Weight of lint in a sample}}{\text{Weight of seed cotton in a sample}} \times 100$$

### Staple Length (mm)

Staple length was measured from the representative sample of each plant using tuft method. The lint sample was turned into sliver and passed it through a draw box for several times till it was drafted into a uniform band of parallel fibres. The fibres were then mounted on a set of metallic comb, fixed parallel to each other on a stand. One of the processed samples was aligned with the help of a pair of tweezers and the two tufts were carefully drawn from each sample on a velvet cover tuft board. Two lines were drawn, one on the even end of the tuft just beneath grip mark of the tweezers and second on the opposite of the tuft, where the rate of change of visual density of the fibre was maximum. The distance between the

**Table 1:** Analysis of Variance for yield of Lint weight.

S.O.V	D. F	S. S	M. S	F. Ratio	P. Value
Replication	2	11.58	5.79	1.35	0.2774
Factor A (Treatment)	4	5935.15	1483.79	346.76	0.0000
Factor B (Varieties)	3	1148.58	382.86	89.47	0.0000
AB (Varieties × Treatment)	12	377.91	31.49	7.36	0.0000
Error	24	102.70	4.28		
Total	59	7691.39			

Grand Mean 40.595; CV 5.10

**Table 2:** Analysis of Variance for GOT %.

S.O.V	D. F	S. S	M. S	F. Ratio	P Value
Replication	2	1.87	0.937	0.98	0.3885
Factor A (Treatments)	4	1068.97	267.241	280.61	0.0000
Factor B (Varieties)	3	22.31	7.435	7.81	0.0008
AB (Varieties × Treatments)	12	65.12	5.427	5.70	0.0001
Error	24	22.86	0.952		
Total	59	1188.69			

Grand Mean 35.251; CV 2.77

two lines was measured with a scale with mm. The average staple length was calculated by taking mean of two readings for the individual plant.

### Staple strength

Strength of the fiber was measured through automatic machine connected to computer software. In this method parallel fibers were obtained by combing and removing extra fiber from the sample then force was applied to cut the fiber and the required force was measured. Greater force represents strength of the fibers.

### Uniformity index

Uniformity index was also measured from the collected samples. In this technique it was observed that the maximum fibers carrying equal lengths.

### Statistical procedures

The data were statistically analysed (Steel and torrie, 1980) for all the levels of significance. For significant F ratios New Duncan’s Multiple Range Test (DMRT) (Leclarg et al., 1962) was applied. The percent increase/decrease in the values were calculated by dividing the difference between the highest and the lowest value over the highest value multiplied by hundred.

## Results and Discussion

### Yield of lint weight

The analysis of variance for Yield of Lint weight indicated significant fluctuation at 5% level of probability (Table 1). The Table of means shows that all the doses of radiations have no significant effect on the yield of lint weight. Table 2 shows that T4 (20 kR) radiation dose produced favourable effect on yield of lint weight with a mean value of 37.3 gm plant<sup>-1</sup> while T5 (25kR) radiation shows minimum yield of lint weight with a value of 28.12 gm plant<sup>-1</sup>. Also the mean values recorded for interaction shows significant differences in varietal performance. The data shows that (Bt CIM 602) produced maximum yield of lint weight with a mean value of 37.49 gm plant<sup>-1</sup> while Bt 131 showed minimum yield of lint weight with a mean value of 30.79 gm plant<sup>-1</sup> (Figure 1).

### Lint percentage (GOT %)

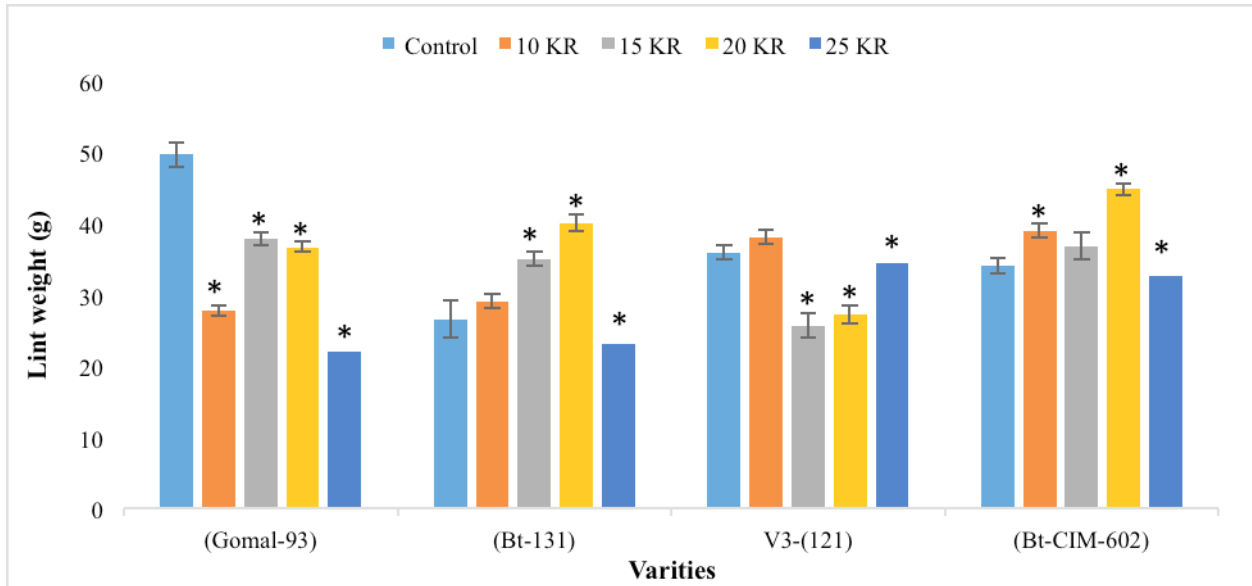
Mean values for plant GOT % of cotton are presented in Table 4. The analysis of variance for GOT % depicted less significant difference among the mean values at 5% level of probability. The mean values revealed less significant difference for GOT % and the varieties (Gomal-93, Bt-131, 121, and Bt-CIM-602) depicted variable response against different doses of

gamma rays from T2 (10 Kr) 37.97 to 35.68 at T5 (25 Kr). Generally a very little decrease in response to higher doses of gamma rays was observed for GOT % in all the varieties (Figure 2).

**Staple length (mm)**

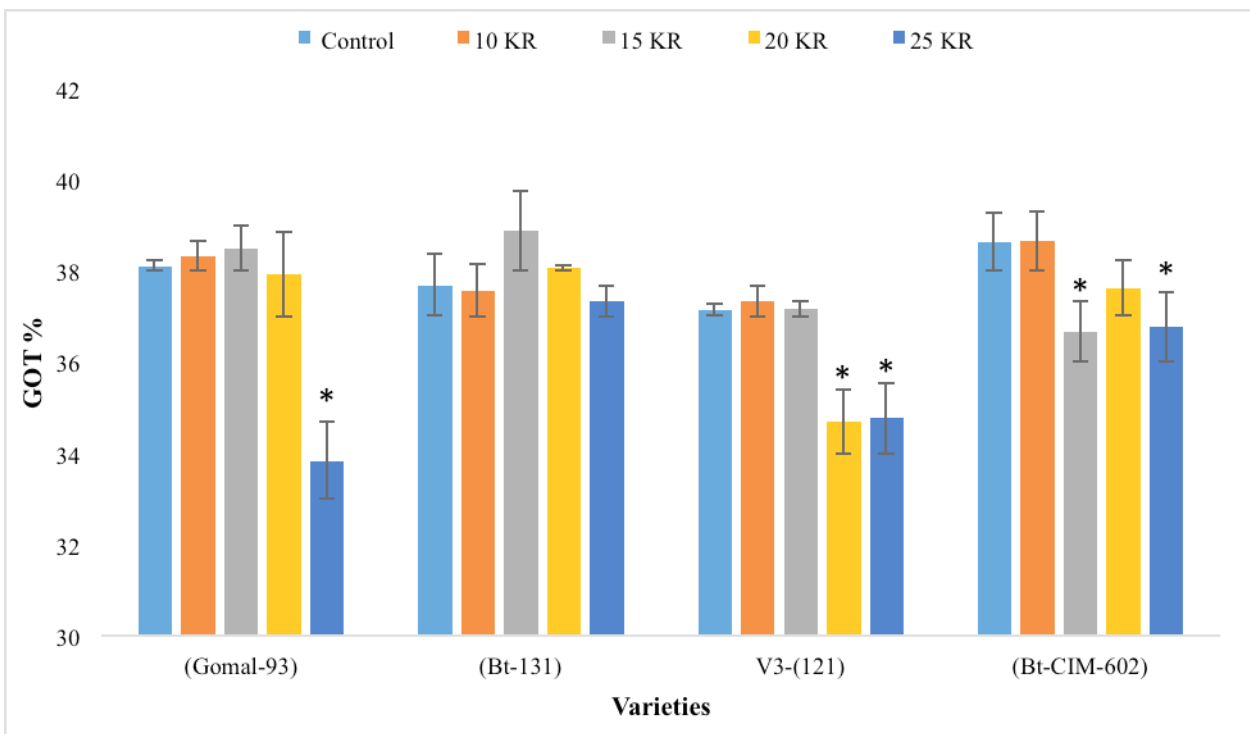
The analysis of variance for staple length revealed sig-

nificant difference in the mean values for staple length (Table 5). The increased Staple length of 29.11 mm was recorded at radiation dose of T5 (25 Kr) while minimum staple length with a mean value of 28.31 mm was recorded at T2 (10 Kr). Table 5 shows minor differences at different doses of radiations. In response to higher doses of radiations the staple length was



**Figure 1:** Comparison of the effects of different treatment of gamma irradiation on lint weight (g) with control using different varieties of cotton.

Error bars shows the standard deviation and asterisks (\*) represent the t test showing the significant of treatment with comparison to control at level of ( $p \leq 0.05$ ).



**Figure 2:** Comparison of the effects of different treatment of gamma irradiation on GOT (%) with control using different varieties of cotton.

Error bars shows the standard deviation and asterisks (\*) represent the t test showing the significant of treatment with comparison to control at level of ( $p \leq 0.05$ ).

**Table 3:** Analysis of Variance for staple length

S.O.V	D. F	S. S	M. S	F. Ratio	P. Value
Replication	2	0.75	0.376	0.43	0.6525
Factor A (Treatment)	4	1182.6	295.658	341.64	0.0000
Factor B (Vrieties)	3	13.53	4.510	5.21	0.0065
AB (Varieties × Treatment)	12	101.99	8.499	9.82	0.0000
Error	24	20.77	0.865		
Total	59	1329.35			

Grand Mean 28.977; CV 3.21

**Table 4:** Analysis of Variance for uniformity index

S.O.V	D. F	S. S	M. S	F. Ratio	P.Value
Replication	2	0.73	0.367	0.19	0.8276
Factor A (Treatment)	4	968.58	242.144	125.75	0.0000
Factor B (Vrieties)	3	34.36	11.452	5.95	0.0035
AB (Varieties × Treatment)	12	32.06	2.671	1.39	0.2383
Error	24	46.21	1.926		
Total	59	1110.53			

Grand Mean 81.648; CV 1.70

**Table 5:** Analysis of Variance for strength

S.O.V	D. F	S. S	M. S	F. Ratio	P.value
Replication	2	2.514	1.257	2.17	0.1360
Factor A (Treatment)	4	834.399	208.600	360.15	0.0000
Factor B (Vrieties)	3	0.756	0.252	0.44	0.7299
AB (Varieties × Treatment)	12	53.702	4.475	7.73	0.0000
Error	24	13.901	0.579		
Total	59	918.417			

Grand Mean 26.173; CV 2.91

increased for almost all the varieties. Regarding varietal performance, maximum staple length of 29.17 was recorded for Gomal-93 while minimum staple length with a mean value of 28.33 was recorded for Bt-131.

### Uniformity index

The analysis of variance for uniformity index at first picking stage illustrates non-significant differences as highlighted in Figure 3 and Table 3. Also the interaction of radiation doses and varieties reflected non-significant differences at 5% level of probability. Figure 3 reveals that maximum uniform numbers of fibers 84.6 were recorded in all the varieties (Gomal-93, Bt-131, Bt -121, and Bt-CIM-602) at T2 (10 Kr). Despite the fact that minimum number of uniform fibers were noted as 82.8 in all varieties (Gomal-93, Bt-131, Bt-121, and Bt-CIM-602) at T5 (25 Kr). Figure 3 indicates descending order from T2 (10 Kr) with 84.6 to 82.8 at T5 (25 Kr). This shows that low doses of

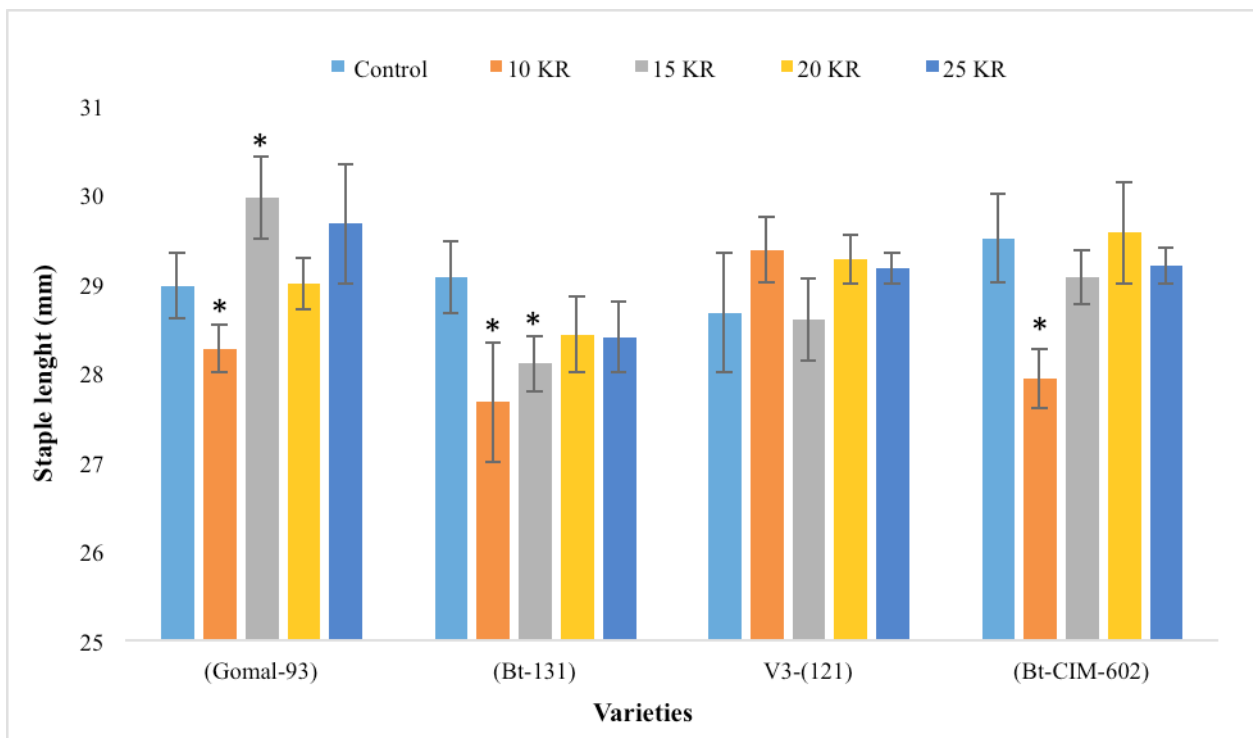
radiation have positive effect on uniformity index. Regarding varietal interaction there was no significant difference between the means value of all varieties. From Figure 3, it is clear that Goaml-93 show maximum number of uniformity index with a mean value of 84.42 while Bt-131 with a minimum mean value 82.56.

### Staple strength (N/Tex)

The analysis of variance for strength at first picking revealed significant differences for the mean values and the interaction between the radiation doses for varietal performance at 5% level of probability as highlighted in Figure 4. Figure 5 presents the mean values for strength and indicated variable effects of radiation doses on different varieties. In case of Gomal-93, increase in the strength over the control (28.67) was observed in response to all the doses except for 10 kR which was recorded 28.37. Bt-121

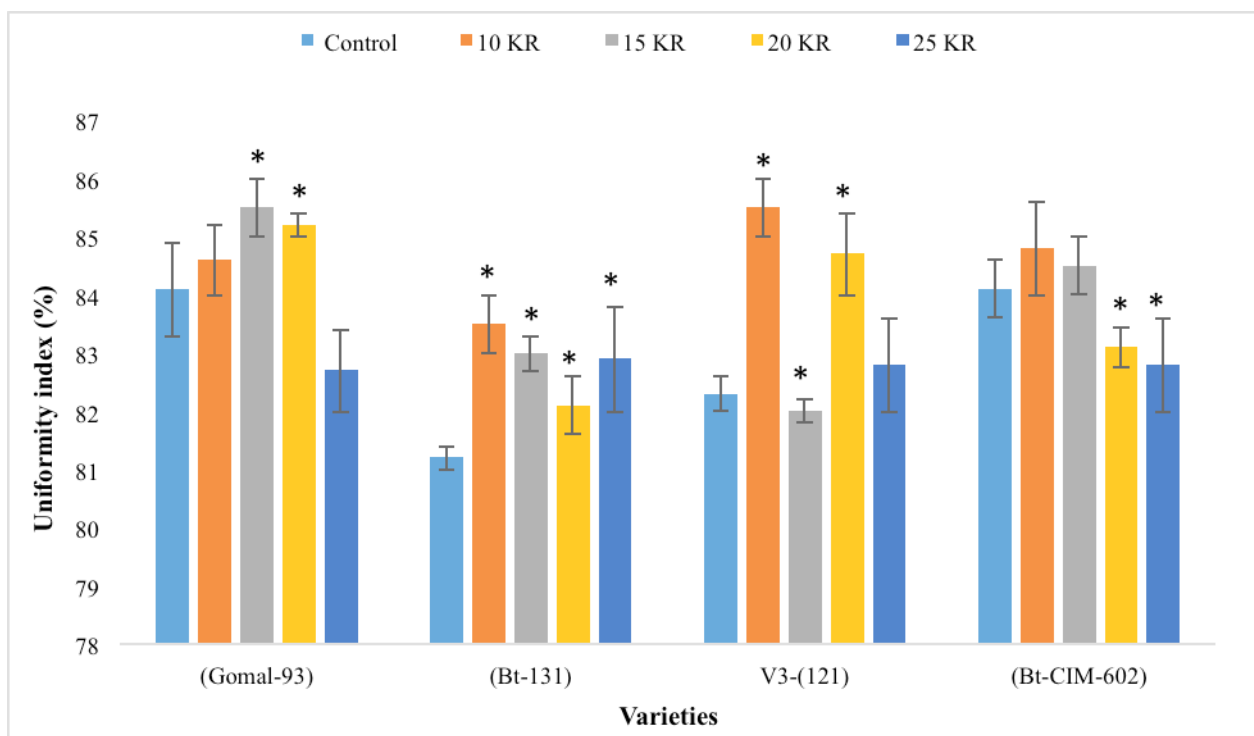
also revealed increase in the strength over the control (28.93) for all the doses of gamma rays and it was in the range of 29.63 to 30.33. Similarly, Bt-CIM-602

also showed increase in the strength over the control (29.10) in response to all gamma rays doses except on 25 kR dose which was recorded as 28.80. Under dose



**Figure 3:** Comparison of the effects of different treatment of gamma irradiation on staple length (mm) with control using different varieties of cotton.

Error bars shows the standard deviation and asterisks (\*) represent the t test showing the significant of treatment with comparison to control at level of ( $p \leq 0.05$ ).



**Figure 4:** Comparison of the effects of different treatment of gamma irradiation on Uniformity index (%) with control using different varieties of cotton.

Error bars shows the standard deviation and asterisks (\*) represent the t test showing the significant of treatment with comparison to control at level of ( $p \leq 0.05$ ).

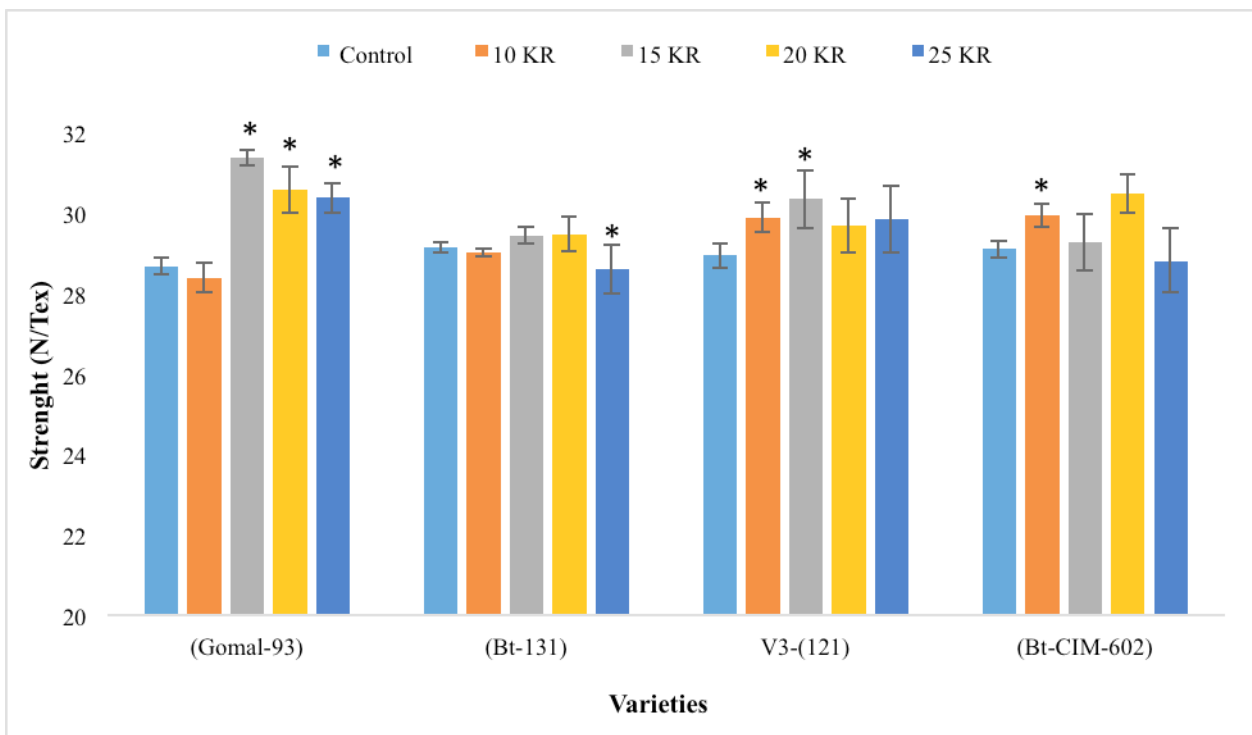
wise average across the varieties, maximum strength of 30.10 was recorded in all the varieties (Gomal-93, Bt-131, Bt-121, and Bt-CIM-602) at radiation dose of 15 kR (T3) whereas minimum strength was noted as 28.95 for all the varieties in control (T1). Non-significant difference between the mean values was observed for interaction among the varieties. The increase and decrease in the strength over the control in response to different radiation doses was non-significant. However, it is evident from the mean values in Figure 5 that different radiation doses have positive effect on the strength of cotton fibres.

The analysis of variance for staple strength at first picking stage illustrates significant differences as highlighted in Figure 5. Also the interaction of radiation doses in varietal performance reflected significant differences at 5% level of probability. Figure 5 regarding mean values of staple length reveals that maximum strength 30.100 was recorded in all varieties (Gomal-93, Bt-131, Bt-121, and Bt-CIM-602) at radiation dose T3 (15 Kr).

Despite the fact that minimum strength were noted as 28.958 in all varieties (Gomal-93, Bt-131, Bt-121, and Bt-CIM-602) at T1 (control). The table of means represent ascending order from control T1 to T3. The

results are matching with those reported by Anderson and Olson (1954), who reported that the increase in yield and oil contents in case of sunflower and white Mustards was due to radiation treatments.

While in varietal interaction there is no significant difference between the mean values of all varieties. Minor difference was found which is non-significant. Gomal-93 with a mean value of 29.867 shows maximum strength while Bt-131 with a mean value of 29.127 shows minimum strength. The table of means represent that radiation has positive effect on the strength of cotton fibres. The Gamma rays are actually ionizing radiations which upon reacting in cell with different atoms or molecules to and generate free radicals. The generated radicals have the potential to alter the critical components in plant cells as well can affect the biochemical, physiological and morphological attributes in plants exposed to gamma irradiation (Wi et al., 2005). Improvement in plants through irradiation needs study to evaluate the improvement in the mutants, developed through treatments with different doses of gamma radiations (Oladosu et al., 2016). Inducing mutations by using gamma radiations have been effectively used in several plant species. Recently, the number of different plant cultivars derived through mutation induction frequently



**Figure 5:** Comparison of the effects of different treatment of gamma irradiation on strength (N/Tex) with control using different varieties of cotton.

Error bars shows the standard deviation and asterisks (\*) represent the t test showing the significant of treatment with comparison to control at level of ( $p \leq 0.05$ ).

increases (Hearn, 2001; Maluszynski et al., 1995; Oladosu et al., 2016).

In the present research work the effect of various treatments of gamma radiation has been investigated in cotton. The various growth parameters like plant height, number of branches, number of bolls, fiber fineness and yield of seed were used as end points.

The results indicated that yield of lint weight was not effected with the application of radiation except T4 (20 KR) radiation which promoted the yield of lint weight. The results are fairly in agreement with those reported by Bridge (2001).

Similarly, the value of plant GOT % revealed less significant difference while the different varieties like Gomal-93, Bt-131, 121, and Bt-CIM-602 respond variably to different doses of gamma rays. Thus the results are somewhat similar to those found by Horvath (1961) who examined the effect of X-rays on *Oryzasativa* L. grown from grains treated in the dry state thereby reduction in germination in response to maximum dose with improvement in straw stiffness and reduction in grain yield.

An increased in staple length was recorded when exposed to the dose of 25 KR while higher doses promoted the staple length in all the tested cotton varieties. The results deviate from those of Penic (1963), who observed reduction in germination and yield in *Oryza sativa* with high doses of radiation. Results regarding the uniformity index revealed that low doses of radiation posed a positive impact on uniformity index. The results are matching with those reported by Anderson and Olson (1954), who examined the effect of X-rays on Sunflower and White mustard which resulted an increase in there oil content. It is evident that different radiation doses have positive effect on the strength of cotton fibres. The results are matching with those reported by Anderson and Olson (1954), who reported that the increase in yield and oil contents in case of sunflower and white Mustards was due to radiation treatments. The results obtained from the present investigations are in agreement with those reported by Anderson and Olson (1954), who reported that the increase in yield and oil contents in case of sunflower and white mustards was due to radiation treatments.

## Conclusion

The agro environment of Bannu is considered out of

the Pakistan commercial cotton belt. The purpose of irradiation was to create desirable mutant that may best adaptable to agro environment of Bannu as well as give ab better yield. It is concluded from the present investigation that cotton seeds when exposed to gamma radiation having different doses, affect the growth parameters of the cotton plants, such as yield of lint weight, lint %, staple length, uniformity index and staple strength. Effects of the different doses of gamma radiations were different on the studied parameters as endpoint as well as different varieties of the cotton showed different responses. Some of the tested varieties showed positive responses to particular dose of gamma radiation and resulted improvement in the growth parameter. It is clear that this technique can be used for production of a mutant with ability to give more yield and have greater adaptability. The findings of this research will be helpful in the improvement of quality and quantity of the cash crops.

## Author's Contribution

SK designed and performed the main experiments and prepared the manuscript. AH and FK helped in performing the experiments and data collection. MS provided supervision, AK and IAS assisted data entry and assisted SKS during manuscript writing. SKS performed the manuscript writing, correction, application of statistics.

## References

- Anderson, G. and G. Olsson. 1954. Svalof's Primex White Mustards a market variety selected in x-ray treated material. *Acts. Agr. Scand.*, 4:574-577. PP. 444-454, "principles of Plant Breeding John Wiley and sons, Inc. New York, London. <https://doi.org/10.1080/00015125409439964>
- Bridge, R.R., J.W.R. Meridith and J.F Chism. 2001. Influence of planting methods and plant population of cotton (*Gossypium hirsutum* L.). *Agro. Res.* 37(5): 104-109.
- Govnenment of Pakistan (GoP). 2014. Agriculture statistics. Pakistan Bureau of Statistics.
- Hearn, C.J. 2001. Development of seedless grapefruit cultivars through bud wood irradiation. *Hort. Sci.* 20: 84.
- Horvat, F. 1961. Radiological investigations on *Oryzasativa*: Effects of x-rays, neutrons and gamma rays. *Agriculture Louvian.* 9: 165-214.
- Irfaq, M. and K. Nawab. 2003. A study to determine



- the proper dose of gamma radiation for inducing beneficial genetic variability in bread wheat (*Triticum aestivum* L.). Asian J. Plant Sci. 13: 999-1003.
- Javed, M.A., A. Khatri, I.A. Khan, M. Ahmad, M.A. Siddiqui, and A.G. Arain. 2000. Utilization of gamma irradiation for the genetics improvement of oriental mustard (*Brassica juncea* Coss.). Pak. J. Bot. 32: 77-83.
- Khatri, A., I.A. Khan, Siddiqui. S. Raza and G.S. Nizamani. 2005. Evaluation of high yielding mutants of *Brassica Juncea* cv. S-9 developed through Gamma rays and EMS. Pak. J. Bot. 37: 279-284.
- Leclarg, R.L., W.H. Leonard and H.G. Clark. 1962. Field plot techniques. 2nd Ed. Burgess Publish. Co. South Minnesota. pp: 144-146.
- Maluszynski, M. 1995. Application of in vivo and in vitro mutation techniques for crop improvement. Euphytica. 85: 303-315. <https://doi.org/10.1007/BF00023960>
- Muller, H.J. 1927. Artificial transmutation of the gene. Science. 66: 84-87. <https://doi.org/10.1126/science.66.1699.84>
- Oladosu, Y., M Y. Rafii, N. Abdullah, N.G Hussin, A Ramli, H.A. Rahim, G.Miah, M Usman. 2016 Principle and application of plant mutagenesis in crop improvement: A review. Biotech Equip. 30: 1-16. <https://doi.org/10.1080/13102818.2015.1087333>
- Penic, M. and D. Jelemic. 1963. Change in some wheat varieties as a result of the effect of Various X-rays doses. Arh. Poljopr. Nauk. 16:19-104.
- Rehman, A., M.L. Das, M.A.R. Howlidar and M.A. Mansur. 1987. Promising mutants in *Brassica campestris*. Mut. Breed. Newsletter. 29: 14-15.
- Smith, C.W. 1995. Cotton (*Gossypium hirsutum* L.), In: Crop Production: Evolution, History and Technology. John Wiley and Sons, Inc., New York. Pp. 287-349.
- Shah S.A., I. Ali and K. Rahman. 2001. Abasin-95 a new oilseed rape cultivar developed through induced mutations. Mut. Breed. Newsletter. 45: 3-4.
- Stadler, L.J. 1928. Mutations in Barley induced by X-Rays and radium. Science. 68: 186-187.
- Steel, R.G.D. and J.H. Torrie. 1980. Principles and procedures of statistics: a biometrical approach. 2nd ed. New York: McGraw-Hill, 633 p.
- Wi, S.G., B.Y. Chung, J.H. Kim. 2005. Ultra-structural changes of cell organelles in Arabidopsis stems after gamma irradiation. J. Plant Biol. 48: 195-200. <https://doi.org/10.1007/BF03030408>