

E.S. Jafarov<sup>1\*</sup> , A.A. Tagiyev<sup>2</sup> , I.Ch. Zeynalova<sup>2</sup> ,  
M.Z. Velijanov<sup>1</sup> , A.E. Jafarov<sup>1</sup> 

<sup>1</sup>Institute of Radiation Problems of the Ministry of Science and Education  
of the Republic of Azerbaijan, Baku, Azerbaijan

<sup>2</sup>Scientific Research Institute of Plant Protection and Technical Plants  
of the Ministry of Agriculture of the Republic of Azerbaijan, Ganja, Azerbaijan

\*e-mail: elimkhan.jafarov@gmail.com

(Received August 18, 2024; received in revised form 19 September 2024; accepted 2 October 2024)

## Comparative analysis of inheritable and modified variations induced by gamma irradiation in the first and second generation of cotton varieties Ganja-160, Ganja-182 and Ganja-183

**Abstract.** The main goal of the conducted research was to obtain cotton genotypes resistant to extreme environmental factors and various diseases, since high doses of  $\gamma$ -irradiation is a mutagenic factor. At the initial stage, before sowing, 1100 samples of plants whose seeds were treated with  $\gamma$ -rays in different doses were cultivated (in four parallel versions), the characteristics of the growing plants were studied, and the plants with changed signs were identified. At the end of the growing season, the raw cotton of 850 plants was collected by individual sampling and the transformed and untransformed plants in M1 were separated, their seeds were collected individually, stored and used for sowing as a family in the next planting (in M2). Changes in vegetation duration, main stem height, number of sympodial branches and number of bolls per bush of both M1 and M2 lineages were evaluated as the main criteria for determining the effectiveness of the mutation. In addition, the main economic characteristics and quality indicators such as the productivity of a bush, fiber yield, and fiber length, mass of raw cotton per boll were determined. It became clear that radiation can create certain changes in the first generation of all three varieties, some of which can be preserved in the second generation. In order to clarify whether the changes observed in the I and II generations are genetic (mutagenic) or just modification changes, the listed parameters are also planned to be studied in the next generations of plants.

**Key words:** Pre-sowing gamma irradiation of seeds, M1 and M2 generations of cotton varieties, economic characteristics and quality indicators.

### Introduction

Today, there is a great demand for new cotton varieties that are productive, fast-growing, resistant to diseases and pests, and have high fiber quality. From this point of view, the creation of new cotton varieties that can provide an intensive increase in cotton productivity and their application in farms is not only an urgent practical issue, but also a matter of scientific importance.

As it is known, one of the most effective methods for obtaining economically valuable starting material in cotton farming is experimental mutagenesis. Experimental mutagenesis is a methodical approach to create cotton varieties with desirable traits and is the best tool to intensify breeding efforts.

It is known that mutation, as well as modification variability, is a characteristic feature of all living things. Mutation can occur in any organism (including plants) even in natural conditions. Mutational variability can also be caused by external influence, more precisely, by changing the influence of external conditions on the organism. For this reason, types of mutagenesis are distinguished, such as natural (or spontaneous) and artificial (or induced).

Today, for obtaining plant genotypes resistant to pests and various extreme factors of the environment, as well as with better quality indicators, selection methods based on artificial, i.e., induced mutations are more preferred [1-4].

This method, called mutation selection, is significantly different in terms of time and cost from

mutations obtained through genetic engineering and allows to create targeted genetic changes. For example, since  $\gamma$ -irradiation accelerates the natural genetic mutation process, the time required for this process is significantly shorter [5]. Besides the vital role in plant breeding programs, a new role of induced mutations in releasing of gene silencing in transgenic plants has been reported [6].

Mutagenesis is known to be a powerful tool for creating new germplasm resources and elucidating the function of plant genes [7, 8]. Artificial mutagenic methods, such as T-DNA insertion mutations and various physical mutagens, have been widely used to create mutations in many types of plants [4].

Until recent years, breeders have preferred to obtain resistant plant forms (hybrids, lines and varieties) based on the use of chemical mutagens. Although these methods give good results, the substances used in some cases have toxic properties, require a lot of labor, and the process is expensive.

It is known that today radiation technologies are widely used in all fields of the national economy, including agriculture. This is due to such advantages as ease of use of radiation technologies, low cost, environmental friendliness, stimulating effect in small doses, high degree of neutralization of planting material, the absence of lethal outcome, minimization of damage to seeds during processing, absence of induced radiation, and reduced energy consumption, etc. For example, exposure of seed to ionizing radiations and the irradiation treatment of male pollen with low doses of gamma rays before cross-pollination resulted in the development of new genetic changes/variability in different crop species [3, 9, 10].

It is believed that one of the main and most common methods for producing mutant varieties is radiation-induced mutagenesis [3, 11, 12].

Gamma rays have both higher linear energy transfer and can transmit more energy to greater depths. For generating mutants, this type of irradiation also has several advantages, such as high mutation efficiency and a wide range of mutations [13].

Radiation mutagenesis is also widely used in cotton breeding. For example, cottonseeds irradiated by  $\gamma$ -rays have been used to obtain heat-resistant and early-maturing mutants [14]. Tong [15] tested cotton mutants for glyphosate resistance through  $^{60}\text{Co}$ - $\gamma$ -ray mutation. In addition, Mu et al. [16] identified cotton mutants with chicken-foot leaves or gossypol-free glands, using  $^{60}\text{Co}$ - $\gamma$ -ray radiation. Chen and Liu [17] found that laser processing of cotton can promote

growth, increase yield, and improve cotton fiber quality.

Because  $\gamma$ -irradiation is a mutagenic factor in high doses, we started research together with well-known cotton growers of our country to buy cotton genotypes that are resistant to adverse conditions and various diseases.

The aim of our research was to obtain mutant cotton lines resistant to various diseases and extreme environmental factors, based on radiation mutagenesis, and use them as a new starting material for selection.

### Materials and methods

Ganja-160, Ganja-182 and Ganja-183 cotton varieties, regionalized by the Republican Agrarian Services Agency, were used as research objects.

Before sowing, cottonseeds were irradiated with Co-60 isotope in doses of 5, 10, 50, 100, 200, 300, 400 Gy (dose rate was 0.342 Rad/sec) in the RUXUND-20000 (Russia) device at the "Isotope Sources of Radiation" Scientific-experimental complex. Non-irradiated seeds of these varieties were used as control options.

Irradiated cotton seeds were sown together with non-irradiated ones (control) in Ganja Regional Agrarian Science and Innovation Center's Samukh experimental base in open field conditions in 90 x 10 cm scheme in 4 replication options (Figure 1).

Considering that experimental mutagenesis did not allow dilution, a limited number of seeds (2 units) were sown in each nest. It is known that dilution in the field violates the percentage of mutation yield and can also destroy plants that have changed in a positive direction, which is important for breeding process. In total, 100 seeds were used for each variant.

During the study, the effect of gamma radiation at different doses on the growth and development of cotton plants was studied by measuring 25 plants in each variant during the phases of mass budding, flowering, and maturation. At the end of the vegetation, the viability of plants was also studied.

Systematic phenological observations were made on plants during the entire vegetation period. At the end of the vegetation, altered plants were recorded in each variant, which differed from the original varieties in terms of phenotypic characteristics. Also, sterile, fertile, etc. forms of the plants have been identified. The collection of raw cotton began with the collection of samples. For this purpose, raw cotton of 20 bolls was collected from I and II places of II-V

bar branches of all plants on each repetition. After the raw cotton of the samples was collected, the modified and unchanged plants were collected as an individual sample. To determine the frequency of mutations, the

number of mutations occurring in 100 families in M2 of a 100-count sample in M1 was studied. Biometric analysis of all indicators, obtained in the study, was calculated according to Dospekhov [18].



Figure 1 – Visibility of the experimental area

## Results and discussion

At the initial stage (in M1) before sowing, plant seeds treated with different doses of  $\gamma$ -rays were cultivated in field conditions; phenological observations and biometric measurements were carried out during the vegetation period. The biomorphological characteristics of the plants were studied, and the plants with changed characteristics were identified. Changes in vegetation duration, main stem height, sympodial branches and the number of bolls per bush are known to be the main criteria that determine the effectiveness of the mutation. Based on what has been said, these parameters, as well as the main economic characteristics and quality indicators, such as the productivity of a bush, cotton fiber yield, fiber length, and the mass of raw cotton per boll, have been determined.

In total, raw cotton of 1100 plants was collected by individual selection, and seeds of transformed and untransformed plants in M1 were collected separately, stored under special conditions and sown as a family in the next planting (in M2). In order to clarify whether the changes observed in M1 are genetic – mutagenic or simply modification changes, it is planned to study the plants not only in M2, but also in the subsequent generations.

We started the research by determining the biomorphological indicators, such as the vegetation period, the length of the plants, the number of bolls and sympodial branches on the bush of the first generation of all three cotton varieties.

The results obtained regarding the duration of vegetation, the length of the plants, the number of bolls and sympodial branches in one bush are reflected in Table 1.

From the results, the first thing that attracts attention is that for all three plants, at doses higher than 200 Gy, the duration of vegetation was about 5-6 days longer compared to the control. It can be considered that radioactive radiation in these doses had an inhibitory effect on the development of plants and slowed down their development.

Of particular interest are the results on plant height. Medium doses of radiation caused the height of plants to increase. On the contrary, high doses of radiation caused them to become shorter.

The number of sympodial branches and the number of bolls per bush also showed a significant dependence on the radiation dose. Apart from minor deviations, it can be considered that the treatment of seeds at high radiation doses (200 Gy and greater) led to an increase in both sympodial branches and the number of bolls per bush. To be more precise, the number of bolls per bush at the radiation doses of 300 and 400 Gy was on average 5 more for Ganja-160, 4 for Ganja-182, and 3 more for Ganja-183 varieties compared to the control.

It was established that plants at higher doses also grew sparsely and developed a large number of side branches from the main stem. At high doses, plants were also characterized by a lack of chlorophyll.

**Table 1** – Dose-dependent change in the bio morphological characteristics of the M1 generation plants whose seeds were subjected to pre-sowing  $\gamma$ -irradiation.

Name of the variety	Radiation doses, Gy	Vegetation period, day	Plant height, cm	Number of sympodial branches	The number of bolls in one bush
GANJA-160	0(C)	122	114.6	13.4	21.1
	5	121	120.3	12.9	21.3
	10	121	117.6	12.8	20.2
	50	120	123.1	12.6	20.5
	100	123	120.8	12.4	19.7
	200	125	120.2	14.3	23.8
	300	128	117.1	15.4	23.7
	400	127	114.4	15.5	26.1
GANJA – 182	0(C)	119	114.6	14.1	19.4
	5	120	104.8	14.4	18.1
	10	119	109.1	13.7	18.5
	50	118	115.1	14.8	21.8
	100	120	118.2	14.6	20.4
	200	121	109.1	14.7	25.1
	300	124	103.1	15.6	24.2
	400	125	103.1	15.5	23.1
GANJA – 183	0(C)	120	112.1	12.8	19.5
	5	119	107.4	11.5	20.1
	10	120	110.1	12.1	19.6
	50	118	123.4	10.7	19.7
	100	122	106.6	11.7	20.2
	200	121	104.6	13.3	23.4
	300	124	100.2	13.4	23.9
	400	126	101.1	13.3	22.8

In general, it was not possible to find any regularity in the change of the height of the main stem and the number of sympodial branches, depending on the radiation dose. Simply, the phenological observations we conducted on cotton varieties showed that despite the normal development of all three varieties at low doses, at doses above 200 Gy the growth of plants slowed down, and they could not give normal output.

Our studies have shown that among the plants changed in M1 at high doses of radiation, there are fast

– growing plants, plants with elongated oval, multi-lobed cones, as well as plants with fruiting organs of 3, 4 and even 5 bolls arranged in a cluster. In the first generation of plants at high doses of radiation, plants with a changed shape were also found, in which the development of the main stem stopped, the lateral branches lengthened, the stem was weak and densely pubescent, the leaves were deeply lobed and heart-shaped, the bush was spreading, the distance between the joints was reduced (Figure 2).



**Figure 2** – The shape of the fruiting organ of plants

Gamma radiation also affected the shape of plant bushes. In fact, at high doses both compact and scattered plants were formed, as well as plants with a strong and branched trunk. At the same time, there were plants with fasciated branches and low-growing plants. At high doses there was a definite change in the shape and number of bolls. Thus, we were able to register plants with grape-shaped, large and small bolls. The effect of gamma radiation on the number and shape of bolls in fruiting organs at high doses has been recorded. Bolls with a hairy, elongated ovoid body and sharp spouts were found at various radiation doses, mainly at doses above 200 Gy.

At the end of the vegetation, in the M1 generation, the changed plants, which differ from the initial varieties in terms of their phenotypic characteristics, were recorded and the sterile, fertile, etc. forms of the plants were determined. Twelve visible phenotypic mutants were observed.

The results of these changes recorded in the M1 generation of cotton varieties treated with  $\gamma$ -rays, as well as the number of late-maturing, early maturing, sterile and semi-sterile plants, are presented in Table 2.

It is clear from the presented results that  $\gamma$ -irradiation can produce different types of variability at different doses.

The effect of  $\gamma$ -radiation on the shape of the bush in plants is more noticeable. Scattered bushy plants are observed in all three cotton varieties at doses of 200 Gy and higher. A radiation dose of 300 Gy in this case produces plants that are more scattered. Plants with strong, branching stems are also preferred at these doses.

It is interesting that if at low doses of  $\gamma$ -irradiation (up to 100 Gy) compact plants were obtained, at high doses (200 Gy and above) both sparsely bushy and highly branched plants were observed.

Plants with branch fasciation were also mostly found at relatively high doses. Such doses, in addition to producing large boll plants, formed clustered boll plants. It has become clear that sterile and semi-sterile plants are also more common in variants corresponding to high values of  $\gamma$ -irradiation dose (doses of 200 Gy and higher). High doses of radiation could also affect the maturity of plants. Therefore, in these doses, the plants matured later.

We believe that the presence of sterile, semi-sterile and chlorophyll-less plants among the changed plants in M1 can be considered a sign of change. Plants with compact form, zero, first and pyramidal branching, as well as fast-growing, high-yielding forms are of special interest for practical selection.

The variability in M1 is assumed to be mainly modification variability. Thus, it is possible to detect rare dominant mutations in M1. Recessive mutations can be observed in M2 after self-pollination. In M1, the seeds of both transformed and untransformed plants, as we already mentioned, were collected separately by individual selection and used for the next planting (for M2). At the end of vegetation, transformed and untransformed plants were also determined for M2 generation plants. In other words, the relevant mutants were screened in M2 generation.

The results (in absolute number and percentage) for the total number of families studied and the number of families changed in both M1 and M2 are presented together in Table 3 for comparison.

**Table 2** – Results on the number and type of variation recorded in the M1 generation of  $\gamma$ -irradiated cotton varieties

The name of the variety	Radiation dose, Gy	Type and number (in pieces) of variation in M1 plants											
		Bush shape					Boll shape			Growing up		Sterile	Semi-sterile
		Compact	Scattered	Plants with strong, branched stems.	Branch fasciation	Short stature	A plant with a cluster-shaped boll	Large	Small	Late growing	Fast growing		
<b>GANJA – 160</b>	0(C)												
	5	1								1			
	10	2			1			1		1			
	50	3											
	100	3			1			1		3			
	200	2	2	5	7			1		5			
	300		4	5	4	2	3	4		3		3	1
	400		3	1			4	5		5		4	3
<b>GANJA – 182</b>	0(C)												
	5	1											
	10	1								2			
	50	2						1		3			
	100	4		2				1		3			
	200	1	3	7	5	3		5		4		1	
	300		4	6				3		3		5	2
	400		1	1	3	3		2		3		3	1
<b>GANJA – 183</b>	0(C)												
	5	2								1			
	10	1							1	2			
	50	2			1		1		3	5			
	100		3		3	2	4	4		3		1	1
	200	1	2	5	2	2		6		5		4	2
	300		3	4	3		3	4				1	
	400		1	1		1	2	2		2		3	2

From the data on the changes caused by gamma irradiation in two consecutive generations of cotton plants, it is clear that the number of changed plants in the M1 generation of plants prevails at high radiation doses. For example, the number of modified plants at irradiation doses of 300 and 400 Gy is 28-42%, 22-33% and 35-70% of the total number of plants for the varieties Ganja-160, Ganja-182 and Ganja-183, respectively.

Some of the changes that occurred in the first generation of plants were also present in their second generation. It is clear from the results that 16 out of 22

plants changed in M1 in Ganja-160 varieties of cotton, according to the radiation dose of 200 Gy kept their changed form in M2. 23 out of 28 plants that changed in M1 in the variant corresponding to 300 Gy radiation dose of this variety and 18 out of 25 plants in the variant corresponding to 400 Gy radiation dose kept their changed form in the next generation.

For Ganja – 182 cotton variety, those figures are 28(21), 23(19) and 17(13) at 200, 300, and 400 Gy radiation doses, respectively, and for Ganja-183 cotton variety, those numbers are again, respectively, 29(21), 21(15) and 14(10).

**Table 3** – Data on the number of changes caused by gamma radiation in M1 and M2 generation plants of cotton varieties

Gamma-radiation dose, Gy	Number of studied families, pieces	Number of families changed in M1		Number of families changed in M2	
		Absolute number	%, ( $\bar{x} \pm S_x$ )	Absolute number	%, ( $\bar{x} \pm S_x$ )
<b>GANJA-160</b>					
0 (C)	316	-	-	-	-
5	296	2	$0.67 \pm 0.47$	1	$0.34 \pm 0.3$
10	292	5	$1.71 \pm 0.76$	3	$0.68 \pm 0.5$
50	292	6	$2.05 \pm 0.83$	4	$0.68 \pm 0.5$
100	268	8	$2.98 \pm 1.04$	5	$1.12 \pm 0.6$
200	220	22	$10.0 \pm 2.02$	16	$3.18 \pm 1.2$
300	100	28	$28.0 \pm 4.49$	23	$15.0 \pm 3.6$
400	60	25	$41.7 \pm 6.36$	18	$13.3 \pm 4.4$
<b>GANJA-182</b>					
0 (C)	356	-	-	-	-
5	352	1	$0.40 \pm 0.34$	1	$0.28 \pm 0.3$
10	336	3	$0.89 \pm 0.51$	2	$0.42 \pm 0.3$
50	292	6	$2.05 \pm 0.83$	5	$0.68 \pm 0.5$
100	288	10	$3.47 \pm 1.08$	7	$1.04 \pm 0.6$
200	272	28	$10.3 \pm 1.84$	21	$4.41 \pm 1.2$
300	104	23	$22.1 \pm 4.07$	19	$8.65 \pm 2.8$
400	52	17	$32.7 \pm 6.50$	13	$11.5 \pm 4.4$
<b>GANJA-183</b>					
0 (C)	336	-	-	-	-
5	308	3	$0.97 \pm 0.56$	2	$0.32 \pm 0.3$
10	316	4	$1.26 \pm 0.63$	2	$0.63 \pm 0.4$
50	276	12	$4.35 \pm 1.23$	7	$1.09 \pm 0.6$
100	240	21	$8.75 \pm 1.82$	15	$3.33 \pm 1.2$
200	224	29	$12.9 \pm 2.24$	21	$6.70 \pm 1.7$
300	60	21	$35.0 \pm 6.16$	15	$13.3 \pm 4.4$
400	20	14	$70.0 \pm 10.25$	10	$20.0 \pm 8.9$

The plants that kept their changed shapes in M2 were also observed for Ganja-182 and Ganja-183 varieties at the radiation dose of 100 Gy. At this radiation dose, the number of plants that retained the altered form in the next generation was 10(7) and 21(15), respectively.

The results show that, in some variants, it was possible to detect the same type of altered plants, recorded in the first generation, in the second generation of plants. A certain part of them was initially assumed to be hereditary. These variations are recorded as mutational variations. The modified cultivar seeds will be used for planting in M3, and

the nature of the variations will be determined. For this reason, those plants were collected separately, according to variants; their number was determined; and it was planned to study them in the third generation to determine whether the variation is hereditary.

The presence of variations in new traits was also found in the second-generation plants. The seeds of the plants with variations in the new traits were also collected separately, and it was planned to use them for planting in M3 and to determine the nature of the variations.

Taking into account that the productivity of one bush, the mass of raw cotton in one boll, fiber yield,

fiber length and strength are important quantitative and qualitative indicators of cotton, we clarified the effect of  $\gamma$ -irradiation on the quantitative and qualitative indicators of both generations of the studied cotton varieties. At the same time, it was also considered that the mentioned indicators may change depending on agro technical measures, abiotic and mutagenic factors. For this purpose, raw cotton of 20 bolls from the I and II places of the second to fifth

sympodial branches at the end of vegetation for both generations of plants was collected by individual sampling, and parameters, such as yield of one bush, mass of raw cotton in one cone, fiber yield, fiber length, were determined.

Table 4 presents the results of the quantitative and qualitative changes observed in the M1 and M2 generations of cotton varieties whose seeds were treated with  $\gamma$ -rays.

**Table 4** – Quantitative and qualitative changes observed in M1 and M2 generations of cotton varieties whose seeds were treated with  $\gamma$ -rays

The name of the variety	Radiation dose, Gy	Productivity of one bush, g		The mass of raw cotton in one boll, g		Fiber yield, %		Fiber length, mm	
		M1	M2	M1	M2	M1	M2	M1	M2
GANJA – 160	0(C)	132.9	126.6	6.3	6.0	34.2	35.0	34.6	33.5
	5	129.9	121.4	6.1	5.7	36.7	36.4	34.9	34.2
	10	127.3	129.3	6.3	6.4	36.4	36.6	33.8	34.0
	50	131.2	125.1	6.4	6.1	36.2	36.6	34.5	32.4
	100	128.1	112.3	6.5	5.7	35.9	34.6	33.6	31.4
	200	145.2	133.3	6.1	5.6	36.5	37.8	33.3	31.6
	300	161.2	146.9	6.8	6.2	35.5	38.3	33.6	31.4
	400	177.5	140.9	6.8	5.4	36.5	38.5	32.3	31.5
GANJA – 182	0(C)	126.1	116.4	6.5	6.0	34.5	35.4	34.1	33.7
	5	119.5	126.7	6.6	6.1	34.8	35.8	34.1	34.4
	10	114.7	112.9	6.2	6.1	34.6	34.9	33.5	33.7
	50	128.6	135.2	5.9	6.2	37.2	37.1	33.7	34.2
	100	126.5	126.5	6.2	6.2	35.4	37.6	33.2	32.5
	200	163.2	130.5	6.5	5.2	34.0	40.2	33.9	32.0
	300	159.7	140.4	6.6	5.8	34.9	39.2	33.4	31.2
	400	143.2	138.6	6.2	6.0	34.0	39.0	34.1	31.3
GANJA – 183	0(C)	126.8	115.1	6.5	5.9	36.6	36.8	34.2	34.5
	5	126.6	116.6	6.3	5.8	37.9	37.7	33.2	32.1
	10	131.3	111.7	6.7	5.7	37.2	38.9	32.6	30.2
	50	130.0	108.4	6.6	5.5	35.7	37.0	33.4	28.4
	100	123.2	123.2	6.1	6.1	34.9	37.2	32.4	30.4
	200	154.4	135.7	6.6	5.8	34.6	32.6	31.7	31.6
	300	153.0	133.8	6.4	5.6	33.7	34.5	33.0	28.7
	400	143.6	125.4	6.3	5.5	33.5	33.1	31.9	31.5

It is clear from the results that the treatment of seeds with  $\gamma$ -rays before sowing can cause certain quantitative and qualitative changes in the M1 generation of cotton, and some of these changes can be preserved in the next generation. A change (increase) in the productivity of a bush is mainly

observed at high doses. For example, if the average yield of one bush was 132.9 g in the control variant of the M1 generation of the Ganja-160 cotton variety, the yield increased at doses of 200, 300 and 400 Gy, and became 145.2, 161.2 and 177.5 g, respectively.



It is interesting that the increase in the productivity of a bush at high radiation doses was preserved in the II generation of this variety. Simply, the difference was that in this case the magnitude of the increase was relatively small.

Another interesting fact is that similar dependence on radiation dose was observed for Ganja-182 and Ganja-183 varieties. In the first generation of the Ganja-182 variety, the mass of cotton per bush was 163.2, 159.7, and 143.2 g at doses of 200, 300, and 400 Gy, respectively (the yield of the control sample was 126.1 g), and the productivity of the Ganja-183 variety at those doses was 154.4, 153.0, and 143.6 g, respectively (the yield of the control sample was 126.8 g). The high yield of cotton in doses of 200, 300 and 400 Gy was maintained in the II generation of these varieties with a slight difference.

In their studies, Muthusamy and Narayanasamy [2] also exposed two varieties of cotton to different doses of  $\gamma$ -rays (at doses of 100, 200, 300, 400 and 500 Gy). Moreover, the selected traits of each mutant also showed higher yield traits in each generation than the parental varieties.

A large number of phenotypic variations was observed in the studies of Zhao et al. [19]. The authors examined three successive generations of cotton and identified variations, including changes in cotton fiber color, plant dwarfing, significant improvements in yields, and increased susceptibility to *Verticillium* wilt. These results indicate that radiation mutagenesis is an effective and feasible method for generating plant mutant libraries.

The high productivity of mutant cotton, obtained as a result of radiation mutagenesis, was also confirmed in the studies of Aslam et al. [3].

Now let's clarify the results we got about the mass of raw cotton of a boll. It is known that the most valuable part of the cotton plant is raw cotton. That is, when we say raw cotton, we understand the cotton seed and the complex of fibers covering it.

The general picture in our results regarding the mass of raw cotton per boll is that for all three cotton varieties, there were no significant changes in the dependence of this parameter on the radiation dose, except for small deviations. Only in one case, a significant increase in the mass of raw cotton of a boll was observed for Ganja-160 variety at 300 and 400 Gy irradiation doses. In these doses, the mentioned parameter was 0.5 g more than the control.

In the second generation of cotton varieties, there was no change in the mass of raw cotton per boll, depending on the radiation dose. In other words, the

mass change that occurred in the I generation was not maintained in the II generation.

Like the mass of raw cotton per boll, fiber yield (the part of the raw cotton that has been separated from the seed) is also an important economic indicator of cotton.

From the results presented in the table, it is clear that the dependence of fiber yield on the radiation dose in the M1 generation is different in different cotton varieties. In fact, fiber yield for Ganja-160 variety was about 1.5-2.5% more than the control at all irradiation doses. In the II generation of this variety, the fiber yield increased more, and the increase was 2.8, 3.3 and 3.5%, respectively, at 200, 300 and 400 Gy irradiation doses compared to the control.

Our results show that there is no significant dependence of the fiber yield of the experimental I generation varieties of the Ganja-182 variety on the irradiation dose. In only one variant (dose 50 Gy), the fiber yield was 2.7% higher than in the control. This result can be considered an experimental error. However, in the II generation of this variety, an increase in approximately the same amount of fiber yield at the same dose gives reason to believe that in this case there may be a stimulating effect. It is interesting that at doses of 200 Gy and higher, there was no obvious dependence of the fiber yield on the radiation dose for the I generation of Ganja-182 variety, but a significant increase in the fiber yield of the II generation of this variety was observed at appropriate doses. More precisely, fiber yield was 4.8, 3.8, and 3.6% higher at 200, 300, and 400 Gy doses than the control, respectively.

The dependence of fiber yield on irradiation dose was completely different for Ganja-183 variety. In fact, in this case, the increase in radiation dose in the first generation of the plant caused a small-scale increase in fiber yield, and then, on the contrary, a decrease. The tendency of fiber output to change in this form was also maintained in the II generation of this variety.

It should be noted that the length of the fiber is one of the most important economically valuable technological characteristics of the cotton plant, and the quality of the fiber is mainly determined by this characteristic. In the textile industry, as a raw material for various types of fabric products, fiber is evaluated according to this indicator.

Like cotton fiber yield, fiber length can also vary under the influence of various agro ecological factors. Changes in water and nutrient regimes, disruption of the agro technological process, and various mutagenic

factors can affect fiber length. The length of the fiber can vary depending on both the variety and the layers of the bolls on the plant.

Table 4, which we present, also shows the results on the dependence of the fiber length on the radiation dose for both generations of all three cotton varieties.

It can be seen from the table that in the first generation of the Ganja-160 variety, there was no change in the dependence of the length of the fiber on the radiation dose in the dose range of 0-50 Gy while a slight decrease trend was observed in the dose range of 100-400 Gy. A similar change occurred in the II generation of this variety. Simply put, the decrease in fiber length was relatively large in this generation. Thus, if in the M1 generation the decrease in fiber length was 1.0, 1.3 and 2.3 mm at doses of 200, 300 and 400 Gy, respectively, then in the M2 generation this decrease was 1.9, 2.0 and 2.1 mm.

Except for small deviations in the length of the fiber of the M1 generation of the Ganja-182 and Ganja-183 varieties, which are within the error of the experiment, almost no change has occurred. However, in the second generation of these cotton varieties, fiber length was shortened at doses of 300 and 400 Gy.

Changes in parameters, such as fiber yield and fiber length in mutant forms of cotton obtained based on the use of radiation technologies, have been confirmed in other works [3, 20-23].

Current work is continuation of research results of which were previously broadcasted in the journal [24].

## Conclusion

Visible phenotypic variations in the amount of 850 plants were selected from 1100 M1 populations at the end of the growing season, using individual selection. Individual selection is of particular importance for the source material, on the basis of which the selection of economically valuable mutant forms will be carried out in subsequent generations.

Seeds of stably inherited mutants were saved for sowing the next generation of M3 cotton. It is also planned to study the M4 and M5 generations of these plants in order to obtain resistant forms to various diseases and environmental stress factors. In other words, we continue our strategy of detecting phenotypic changes. We hope that radiation mutagenesis, which is an effective and feasible

method for creating libraries of plant mutants, will make it possible to obtain cotton mutants with stable heritable traits.

The selection of more valuable forms will be carried out by studying the observation of a single mutant trait or a complex of positive traits in a hybrid generation. Selected mutant hybrids will be studied for their breeding and agricultural value. According to the characteristics of mutation selection, stable mutants with one or more selection characteristics preserved in subsequent generations will be crossed with the original varieties (or with each other). That is, hybridization will be carried out according to the accepted method.

It is clear that the creation of mutant forms of cotton is of great practical importance since this allows for genetic improvement of cotton and the creation of new varieties. It should be noted that although traditional breeding methods have made a great contribution to the cultivation of cotton varieties, they have led to significant loss of genetic potential and increased susceptibility to pests [25]. Therefore, expansion of the genetic background through mutations can diversify functional genes, create new traits, and generate more germplasm resources [26]. In addition, a library of mutants is of great importance for studying gene function since acquired traits, such as fiber length, stem height, harvest quantity, leaf morphology, fiber color and other mutants, can greatly contribute to genetic breeding and basic research in cotton [19].

Summarizing the data we obtained from two generations of cotton, we can conclude that, in general, radiation produced many types of mutants, some of which had positive traits. We suggest that these forms can be used as germplasm to improve the properties of cotton.

## Acknowledgements

This work was carried out on the basis of the State Program designed for 5 years (2021-2025) on the topic "Obtaining productive varieties of cotton with high quality indicators and resistant to extreme environmental factors using radiation technologies."

## Conflict of interest

All authors are aware of the article's content and declare no conflict of interest.

## References

1. Aslam M.Z. (2002). Evolution of high yielding, early maturing and CLCuV resistant mutant of cotton NIAB-98, through the use of pollen irradiation approach. *Plant Pathology Journal*, 1(1), pp. 27-31. <https://doi.org/10.3923/ppj.2002.27.31>.
2. Muthusamy A., Narayanasamy J. (2005). Induced high yielding mutant in cotton (*Gossypium hirsutum* L.). *Mutation Breeding Newsletter and Reviews*, 1(1), pp. 6-8. <https://www.researchgate.net/publication/235766382>.
3. Aslam M.Z., Haq M.A., Bandesha A.A., Haidar S. (2018). NIAB-846: high yielding and better quality cotton mutant developed through pollen irradiation technique. *Pakistan Journal of Agricultural*, 55(4), pp. 767-776. <https://doi.org/10.21162/PAKJAS/18.5133>.
4. Liu J., Zhao G., Geng J., Geng Zh., Dou H. et al. (2023). Genome-wide analysis of mutations induced by carbon ion beam irradiation in cotton. *Frontiers in Plant Science Front (Sec. Plant Breeding)*, 14. <https://doi.org/10.3389/fpls.2023.1056662>.
5. Maluszynski M. (1990). Gene manipulation in plant improvement. II. (Gustafsson J.P., ed.), New York. Plenum press, 438 p.
6. Bhatia C.R. (1999). Release of gene silencing in transgenics – a new role for induced mutations. *Mutation Breeding Newsletter & Reviews*, 44, pp. 3-5.
7. Holme I. B., Gregersen P. L., Brinch-Pedersen H. (2019). Induced genetic variation in crop plants by random or targeted mutagenesis: Convergence and differences. *Frontiers in Plant Science*, 10. <https://doi.org/10.3389/fpls.2019.01468>.
8. Bhoi A., Yadu B., Chandra J., Keshavkant S. (2022). Mutagenesis: A coherent technique to develop biotic stress resistant plants. *Plant Stress*, 3 (100053). <https://doi.org/10.1016/j.stress.2021.100053>.
9. Iqbal R.M.S., Chaudhry M.B., Aslam M. and Bendasha A.A. (1994). Development of a high yielding cotton mutant NIAB-92 through the use of induced mutations. *Pakistan Journal of Botany*, 26, pp. 99-104.
10. Maluszynski M., Ahloowalia B.S. and Sigurbjornsson B. (1995) Application of in Vivo and in Vitro Mutation Techniques for Crop Improvement. *Euphytica*, 85, pp. 303-315. <https://doi.org/10.1007/BF00023960>.
11. Ishikawa S., Ishimaru Y., Igura M., Kuramata M., Abe T. et al. (2012). Ion-beam irradiation, gene identification, and marker-assisted breeding in the development of low-cadmium rice. *Proceedings of the National Academy of Sciences*, 109(47), pp. 19166-19171. <https://doi.org/10.1073/pnas.1211132109>.
12. Kazama Y., Hirano T., Nishihara K., Ohbu S., Shirakawa Y., Abe T. (2013). Effect of high-LET Fe-ion beam irradiation on mutation induction in *Arabidopsis thaliana*. *Genes & Genetic Systems*, 88(3), pp. 189-197. <https://doi.org/10.1266/ggs.88.189>.
13. Permata T.B.M., Sato H., Gu W., Kakoti S., Uchihara Y. et al. (2021). High linear energy transfer carbon-ion irradiation up regulates PD-L1 expression more significantly than X-rays in human osteosarcoma U2OS cells. *Journal of Radiation Research*, 62(5), pp. 773-781. <https://doi.org/10.1093/jrr/rrab050>.
14. Toker C., Yadav S.S., Solanki İ. (2007). Mutation Breeding (In book: Lentil), pp. 209-224. [https://doi.org/10.1007/978-1-4020-6313-8\\_13](https://doi.org/10.1007/978-1-4020-6313-8_13).
15. Tong X. H. (2021). Selection and Mechanisms of Glyphosate to Lerant Mutant R0198. Zhejiang University.
16. Mu G.J. (2008). Creation and Molecular Genetical Identification of the Beneficial Mutants in Upland Cotton (*Gossypium hirsutum* L.). Hebei Agricultural University.
17. Chen Z.G., Liu X.G. (1993). Effect of CO<sub>2</sub> laser water pretreatment on isolated cotyledon culture of cotton. *Journal of Anhui Agricultural University*, 20(1), p.3.
18. Dospekhov B. A. (1985). Field experiment method [Metod polevogo opyta]. M.: Agropromizdat, 352 p.
19. Zhao Z., Liu Z., Zhou Y., Wang J., Zhang Y. et al. (2022). Creation of cotton mutant library based on linear electron accelerator radiation mutation. *Biochemistry and Biophysics Reports*, 30, 101228. <https://doi.org/10.1016/j.bbrep.2022.101228>.
20. Muthusamy A. and Jayabalan N. (2011). In vitro induction of mutation in cotton (*Gossypium hirsutum* Linnaeus) and isolation of mutants with improved yield and fibre characters. *Acta Physiologiae Plantarum*, 33, pp. 1793-1801. <https://doi.org/10.1007/s11738-011-0718-8>.
21. Muhammad A., Wazir S.M., Ullah H. and Afridi S. (2015). Effect of Selected -Irradiated Cotton Varieties on Fiber Quality During M2 Generation under Rainfed Condition. *American-Eurasian Journal of Agricultural & Environmental Sciences*, 15(2). pp. 191-196. <https://doi.org/10.5829/idosi.aejaes.2015.15.2.12511>.
22. Haidar S., Aslam M. and Haq M.A. (2016). NIAB-852: Anew high yielding and better quality cotton mutant developed through pollen irradiation technique. *Pakistan Journal of Botany*, 48(6), pp. 2297-2305. <https://www.researchgate.net/publication/312495225>.
23. Orabi M.H., El-Hoseiny H.A., Abd-El-Rahman Y. Sh., Khater M. S. (2017). The Effect of Gamma Rays on Cotton Yield, Yield Components and Fiber Quality Characters. *Journal of Plant Production*, 8(12), pp. 1277-1284. <https://doi.org/10.21608/JPP.2017.41981>.
24. Zeynalova I.C., Tagiyev A.A., Gojayeva G.A., Jafarov E.S. (2022). types and economically valuable features of change produced by the gamma radiation before sowing the seeds by the M1 generation of the cotton plant?. *International Journal of Biology and Chemistry*, 15(2), pp. 40-46. [doi.org/10.26577/ijbch.2022.V15.İ2.06](https://doi.org/10.26577/ijbch.2022.V15.İ2.06).
25. Aslam U., Cheema H., Sheraz A., Khan, I.A., Waqas M., Khan A.A. (2016). COTIP: cotton-TILLING platform, a resource for plant improvement and reverse genetic studies. *Frontiers in Plant Science*, 7, 1863. <https://doi.org/10.3389/fpls.2016.01863>.
26. Xu T., Bian N., Wen M., Xiao J., Yuan C. et al. (2017). Characterization of a common wheat (*Triticum aestivum* L.) high-tillering dwarf mutant. *Theoretical and Applied Genetics*, 130, pp. 483-494.

**Information about authors:**

*Elimkhan Jafarov – (corresponding author) – Doctor of Science, Professor, Institute of Radiation Problems of the Ministry of Science and Education of the Republic of Azerbaijan, Baku, Azerbaijan, e-mail: elimkhan.jafarov@gmail.com*

*Aladdin Tagiyev – Doctor of Science, Professor, Scientific Research Institute of Plant Protection and Technical Plants of the Ministry of Agriculture of the Republic of Azerbaijan, Ganja, Azerbaijan, e-mail: t.eleddin@mail.ru*

*Intizar Zeynalova – Associate Professor, Scientific Research Institute of Plant Protection and Technical Plants of the Ministry of Agriculture of the Republic of Azerbaijan, Ganja, Azerbaijan, e-mail: z-va.tarana@mail.ru*

*Mehriban Velijanova – PhD, Associate Professor, Institute of Radiation Problems of the Ministry of Science and Education of the Republic of Azerbaijan, Baku, Azerbaijan, e-mail: mehriban.velijanova@gmail.com*

*Anar Jafarov – Associate Professor, Institute of Radiation Problems of the Ministry of Science and Education of the Republic of Azerbaijan, Baku, Azerbaijan, e-mail: anar\_jafarov@internet.ru*