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Original Research

Mutagenic Variations in Four Successive Generations of Cotton Varieties from Gamma Ray Treated Seeds

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Abstract

Seeds of cotton varieties Ganja-160, Ganja-182, and Ganja-183 were γ-irradiated with the isotope Co-60 at doses of 5, 10, 50, 100, 200, 300, and 400 Gy (at a dose rate of 0.342 rad/sec). Irradiated seeds, along with non-irradiated seeds (controls), were sown at the experimental base of the Center for Agricultural Sciences under open-field conditions in four replicates. The aim was to obtain mutant cotton lines with high quality and improved technological parameters, as well as resistance to various diseases and extreme environmental factors, and to use them as starting material for breeding. The nature of phenotypic changes occurring in four successive generations obtained by radiation mutagenesis was studied, and the economically valuable characteristics of mutant forms and fiber quality parameters were determined. Based on changes across four successive generations of plants, it was possible to identify mutant forms with 46 economically important traits, such as productivity, high fiber yield, and long, strong fiber, for breeding purposes. The results also showed that these plants not only exhibited high productivity but also had improved fiber parameters, such as fiber length and strength. The mutant cotton lines also exhibited improved technological parameters, such as fiber linear density, breaking strength, and staple length. The results demonstrated that γ- irradiation of seeds not only resulted in numerous phenotypic changes, high genetic diversity, and a wide spectrum of mutations in subsequent generations of these plants, but also enabled the development of mutant forms with several economically important traits. Based on these results, it can be concluded that radiation mutagenesis is an effective method for creating mutant plant libraries.

Keywords

Radiation mutagenesis; M1, M2, M3 and M4 generations of cotton; quality and technological parameters

1. Introduction

Cotton (*Gossypium* spp.) is one of the most important commercial crops in the world. Increase in cotton yields while simultaneously improving fiber quality remains the main task for geneticists and breeders [1]. It should be noted that the number of bolls per plant, fiber yield, lint percentage, the weight of raw cotton in a single boll, and the number of plants per unit area determines cotton productivity. In contrast, fiber quality is characterized by a set of indicators, including fiber length, strength, and fineness, as well as technological parameters such as linear density, relative breaking load, and staple length. The combination of these quantitative and qualitative indicators significantly improves cotton's competitiveness.

There is a strong need to develop new cotton varieties with high yields, rapid growth, disease and pest resistance, and high fiber quality. It is known that new varieties of cotton have been obtained mainly by traditional methods of crossing, which were limited not only by the available genetic material but also were time-consuming [2, 3]. It has not yet been possible to detect a positive correlation, and a negative correlation is generally observed between yield and fiber quality. In other words, it has not yet been possible to simultaneously improve these two traits using traditional selection methods [4]. For this reason, efforts are being made to create mutants of this plant to obtain new, improved cotton varieties and to identify genes, regulating key traits through induced mutations [3, 5]. This method, called mutation breeding, differs significantly in terms of time and cost from genetic engineering approaches and allows for the creation of targeted genetic changes [6, 7].

Induced mutagenesis is a known powerful tool for generating new genetic resources and elucidating the functions of plant genes [8, 9]. Artificial mutagenic methods such as T-DNA insertion mutations and various physical mutagens have been widely used to create mutations in many plant species [10, 11].

Experimental mutagenesis is one of the most effective methods for obtaining economically valuable source material [6, 12]. Experimental mutagenesis is a methodical approach for developing cotton varieties with desired characteristics and is the best tool for intensifying breeding work [13].

It should be noted that until recently, plant breeders preferred chemical mutagens to create plants (hybrids, lines, and varieties) that were not only high-yielding but also resistant to various diseases and extreme environmental factors. Although these methods have yielded good results, the substances used were often toxic, and the process itself was labor-intensive and expensive [14]. Some traditional physical mutagenic strategies and site-directed mutagenesis used in this case were also environmentally unfriendly, relatively expensive, inefficient, and difficult to implement [15].

Seeds, as is known, are the carriers of the biological properties of plants. In this regard, the quantitative and qualitative indicators of the obtained product depend significantly on the condition of the seeds. The need to process the seeds with various methods before sowing also helps stimulate processes within them, thereby improving germination and seedling vigor. Gamma radiation at certain doses can excite the embryo and increase enzymatic activity, thereby increasing the rate of cell division, can increase the rate of not only germination but also vegetative growth [16]. Radiation mutagenesis has wide application in this regard, since it is based on a more efficient and environmentally friendly technology. Currently, radiation technologies are widely used in all sectors of the national economy, including agriculture [17-19]. This is because radiation technologies have advantages, such as ease of use, low cost, environmental friendliness, a stimulatory effect at low doses, mutagenicity in certain doses, a high degree of neutralization of planting material and absence of lethal outcome, as well as minimization of seed damage during processing, absence of induced radiation, and reduced energy costs [20, 21].

Ionizing radiation has been used for nearly 70 years to create new varieties with high economic value, resistance to pests and diseases, high yields, improved quality, higher nutritional value, and other novel traits [13, 22-24]. For example, exposure of seeds to ionizing radiation and irradiation of male pollen with low doses of gamma rays before cross-pollination induced new genetic changes in different crop species [25-28].

The aim of this study was to investigate the effect of pre-sowing treatment of seeds with gamma rays on the quality indicators and technological parameters of cotton plants, as well as to obtain mutant cotton lines based on radiation mutagenesis that are resistant to various diseases and extreme environmental conditions, and to use them as a new source of material for breeding.

2. Materials and Methods

Cotton varieties Ganja-160, Ganja-182 and Ganja-183 were the genetic materials used. Ganja-160 is more productive, drought-resistant and has a high-quality fiber that meets the needs of the textile industry. Ganja-182 is an early-maturing variety with a 115-day vegetative period.

Compared to Ganja-160, its stem is compact; cones are located closer to the stem and are very suitable for mechanized cleaning. Ganja-183 is distinguished by a high fiber yield (42%) and exhibits both types of branching. All three varieties are adapted to local soil and climatic conditions.

Prior to sowing, cotton seeds were gamma-irradiated with a Co-60 source at doses of 5, 10, 50, 100, 200, 300, and 400 Gy (at a dose rate of 0.342 rad/sec). Irradiation was performed using a RUXUND-20000 facility at the Research and Experimental Complex “Isotope Radiation Sources” of the Institute of Radiation Problems of the Ministry of Science and Education of Azerbaijan. Non-irradiated seeds of these varieties were used as control samples. Both irradiated and non-irradiated (control) seeds were sown at the experimental base of the Ganja Regional Center for Agricultural Science and Innovation in an open field using a 90 × 10 cm planting scheme, with four replicates. According to the principles of experimental mutagenesis, a limited number (two) of seeds were sown per hill. Deviation from this condition may lead to the loss of plants exhibiting positive changes, thereby reducing the overall mutation frequency. A total of 100 seeds were used for each treatment variant.

Systematic biometric measurements and phenological observations were conducted on plants throughout the vegetation period. At the same time, the influence of gamma radiation at different doses on the growth and development of cotton was studied by measuring 25 plants per variant. Measurements were carried out at the stages of mass budding, flowering and ripening.

At the end of the growing season, untreated cotton from modified and unmodified M1 plants was collected using individual selection. Phenotypically modified samples were particularly valuable as source material, as they were used to select economically valuable mutant forms in subsequent generations. Seeds from these accessions were stored under special conditions and sown in separate groups for the next sowing (for the M2 generation). Phenotypic variations were observed in the second generation. At the end of the growing season, transformed and non-transformed plants of the M2 generation were also identified. To determine whether the variation was heritable, these plants were also collected separately depending on the variant. In other words, the modified families of the M2 generation were separated by individual selection and considered as mutant forms. To understand the genetic nature of the transformed families, seeds from these plants were used as planting material for the next M3 generation. Phenotypic changes occurring in plants were studied in the same way in the fourth generation. Also, biometric analysis of all parameters obtained during the study was conducted according to Dospekhov [29]. The results were statistically processed using standard methods of Analysis of Variance. The difference between the means obtained for the irradiated and non-irradiated (control) plants was assessed using the Student's t-test [30]. The reliability of the differences was considered significant at $|t| > 2$ ($p < 0.05$).

3. Results

In the initial stage of the study (in M1 generation plants), seeds treated with γ-rays at different doses prior to sowing were grown in the field, systematic observations were conducted, and plants with altered forms were identified. It was clear that irradiation led to the formation of many branching variants in the plants. Such branching diversity was more pronounced at high doses. High-dose irradiation led to the formation of numerous lateral branches on the main plant stem. Our observations showed that high doses of irradiation also affected the number and shape of bolls. Thus, bulbs of various types and shapes and fruiting organs of various shapes were observed. At high doses of radiation, excessively large or small bolls, as well as elongated, polygonal, pointed and oval-shaped bolls were found. Some plant variants had dozens of small bolls on short sympodial branches. Clusters of bolls were also observed. Such forms are considered rare genetic variants.

At the end of the vegetative phase, altered plants that differed from the original varieties in phenotypic trraits were identified. Non-chlorophyllous, sterile, and fertile forms of the plants were also identified. In other words, the M1 plants were divided into transformed and non-transformed groups based on parameters that determine mutation efficiency. These parameters included the duration of the growing season, the size of the main stem and sympodial branches, and the number of bolls per plant. Other parameters considered were bush shape (compact, spreading, robust, branched, branch fasciation, dwarfism), boll shape (large, small, and grouped), ripening time (early, late), sterility, and semi-sterility.

Results on the number of transformed forms, as well as the dependence of morphological traits on radiation dose, showed that plants grown from irradiated seeds differed in their phenotypic traits. Moreover, increasing the gamma irradiation dose of seeds significantly increased the number of transformed forms for all cotton varieties in the M1 generation. More precisely, in the range of radiation doses of 5-50 Gy, the number of transformed plants ranged from 2 to 12, whereas in the range of doses of 100-400 Gy, it varied from 10 to 30. With an increase in the dose of gamma irradiation, the growing season of all three cotton varieties also increased. High doses, by suppressing plant development, extended the growing season by approximately 4-6 days. High doses also increased both the number of sympodial branches and the number of bolls per plant.

The presence of sterile, semi-sterile, and non-chlorophyllous plants among M1 plants can be considered a sign of variability. Of particular interest for practical breeding were plants with compact forms, without primary and pyramidal branches, and early-ripening, high-yielding varieties.

At high doses, mainly late-ripening plants were observed, while at relatively low doses, early-ripening plants were observed.

Considering that the yield of a bush, the mass of raw cotton per boll, fiber yield, fiber length, and fiber strength are the main indicators of cotton, the dependence of these economically important indicators on gamma radiation dose was also clarified for 850 plants in which changes in M1 were detected.

The study of morphological changes in the second generation also identified transformed and non-transformed plants. Moreover, with increasing radiation dose, the number of transformed plants increased in all cotton varieties. The difference is that the number of modified plants in the second generation was approximately 30-35% lower than in M1-generation plants. In other words, not all modified forms are retained in the next generation. Only 60-65% of the changes that occurred in the first generation are retained in the second generation.

Quantitative and qualitative indicators, such as yield per plant, raw cotton boll weight, fiber yield, and fiber length, were also determined for the second generation cotton. The results showed that branching patterns, boll size, fiber yield and length, productivity, growing season, main stem length, etc., are heritable in this generation. As expected, the same modified forms were observed in various variants in the second-generation plants as well. The modified families of the M2 generation were considered mutant forms.

Our study continued by examining M3 generation plants grown from seeds of transformed M2 generation plants. We also determined the number of transformed plants for this generation.

The results for the number of families that changed in the M2 generation and progressed to the M3 generation are presented in Table 1.

Table 1 Number of families that changed in the M2 generation and used in the M3 generation.

Variety name	Radiation doses (Gy)	Number of families	Families changed in M2		Families genetically transformed to M3	
			Number	**%**	**Number**	**%**
Ganja-160	0 (C)	316	-	-		
	5	296	12	4.1	5	41.7
	10	292	15	5.1	6	40.0
	50	292	16	5.5	6	37.5
	100	268	18	6.7	10	55.6
	200	220	28	12.7	26	92.9
	300	100	31	31.0	29	93.6
	400	60	31	51.7	28	90.3
Ganja-182	0 (C)	356	-	-	-	-
	5	352	13	3.7	4	30.8
	10	336	17	5.1	5	29.4
	50	292	16	5.5	8	50.0
	100	288	21	7.3	11	52.4
	200	272	38	14.0	29	76.3
	300	104	36	34.6	21	58.3
	400	52	27	51.9	22	81.5
Ganja-183	0 (C)	336	-	-	-	-
	5	308	13	4.2	6	46.1
	10	316	14	4.4	6	42.9
	50	276	22	8.0	15	68.2
	100	240	31	12.9	28	90.3
	200	224	39	17.4	28	71.8
	30	60	31	51.7	25	80.7
	400	29	24	82.8	23	95.9

Results from studies of three consecutive cotton generations showed that increasing the gamma radiation dose leads to an increase in both the number of mutant families in the M2 generation and the mutation frequency in the M3 generation. As hown in the table, gamma irradiation produced transformed plants in all three cotton varieties. The number of transformed plants depends not only on the cotton variety, but also on the radiation dose. Although there are no significant differences among varieties, differences in response to radiation dose are clearly visible. More precisely, an increase in radiation dose results in a greater number of transformed plants. The increase in the number of transformed plants is more pronounced at radiation doses of 100-300 Gy than at lower doses.

Phenotypic changes occurring in plants were also studied in the fourth generation. The data on these changes are presented in Table 2.

Table 2 Number of transformed plants in the M4 generation that differed from the initial varieties in terms of phenotypic characteristics.

Variety name	**Radiation doses (Gy)**	**Number of studied families**	**Family changed in M4**	
			Number	**%, (x ± Sx)**
Ganja-160	0 (C)	-	-	-
	5	5	2	40.0 ± 8.7
	10	6	4	66.7 ± 18.4
	50	6	5	83.3 ± 21.4
	100	10	7	70.0 ± 16.6
	200	26	17	65.4 ± 14.7
	300	29	24	82.8 ± 24.1
	400	28	22	78.6 ± 17.2
Ganja-182	0 (C)	-	-	-
	5	4	3	75.0 ± 12.8
	10	5	4	80.0 ± 15.2
	50	8	5	62.5 ± 12.4
	100	11	6	54.5 ± 17.1
	200	29	24	82.8 ± 18.9
	300	21	17	81.0 ± 14.3
	400	22	16	72.7 ± 17.1
Ganja-183	0 (C)	-	-	-
	5	6	4	66.7 ± 12.1
	10	6	4	66.7 ± 11.8
	50	15	13	86.7 ± 20.3
	100	28	21	75.0 ± 13.4
	200	28	27	96.4 ± 21.4
	300	25	23	92.2 ± 17.9
	400	23	21	91.3 ± 17.1

The number and type of changes occurring in the fourth-generation plants are presented in Table 3.

Table 3 Number and types of changes occurring in the M4 generation of the studied plants.

Variety name	Radiation doses (Gy)	Type and number of plants changed in M4											
		Shape of the bush					Shape of the bolls			Maturity		Sterile	Semi - sterile
		Compact	Scattered	Plants with strongly branched stems	Branch fasciation	Dwarfism	Clustered bolls	Large	Small	Late - maturing	Early - maturing		
Ganja-160	0 (C)												
	5	1		2				1			1		
	10	2						1			1		
	50	4		2				1			1		
	100	1	3		1				1	1			
	200		3		4		1	2			4	1	
	300	2	5		4	3	3	3				4	
	400		5	2				3	4	3		3	2
Ganja-182	0 (C)												
	5	3		2				1			2		
	10	2								1	2		
	50	2		1	1			2			3		
	100	1		1	2		1	1		1	1		
	200		4	1	4	4	3	3	1	4		1	
	300		5	1	4	3	2		1	2		4	1
	400		3		2	3	1		1	3		4	2
Ganja-183	0 (C)												
	5	4		2				1			2		
	10	2		1				1			2		
	50	3		2				2			6		
	100		2		4		2		3	5		2	1
	200		4	1	3	3	3	3		6		3	
	300		5	2	4	2	4		5		1	2	1
	400		4		3	2	3		3	4		4	2

Plants with scattered, highly branched stems, branch fasciation, and bushy forms, such as dwarf plants, were also primarily observed at high irradiation doses. In this case, changes in boll shape (large bolls, clustered bolls) were also observed. High irradiation doses also resulted in the formation of sterile and semi-sterile plants. In addition, some plant variants exhibited a wide range of mutations. In particular, these variants possessed 4-5, or even 6 economically important traits, such as large bolls, compact bushes, highly productive, fast-growing plants with high fiber yield, tall stems and resistance to wilting. Based on these traits, we selected 113 mutant forms from the M4 generation of plants. With different radiation doses, it was possible to obtain 35, 41 and 37 mutant forms for the varieties Ganja-160, Ganja-182 and Ganja-183, respectively. However, forms with economically significant traits were detected primarily in the 5-50 Gy dose range. At higher doses, the number of such mutant forms was small, and at 400 Gy, it was impossible to obtain forms with economically significant traits.

Mutation sources and their numbers, depending on cotton variety and radiation dose, are presented in Table 4.

Table 4 Mutation sources and their numbers depending on cotton variety and radiation dose.

Radiation doses (Gy)	**Number of mutant forms obtained in M4**		
	Ganja-160	**Ganja-182**	**Ganja-183**
0 (C)	-	-	-
5	6	12	9
10	9	11	9
50	10	10	6
100	7	3	5
200	3	4	7
300	-	1	1
400	-	-	-
Total	35	41	37
	113		

As is known, each mutant form with economically important traits is considered a valuable starting material for crossing. In this regard, the donor properties of the best indicators of mutant forms obtained by radiation mutagenesis have been studied and grouped.

Considering that improving processing parameters such as fiber linear density, relative breaking load, and staple length increases the competitiveness of cotton, we studied these parameters. Quantitative and qualitative indicators such as maturation period, productivity, fiber yield, fiber productivity, fiber length, and boll mass, have also been determined for the obtained mutants.

The results are presented in Table 5. Productivity of the mutant form of the Ganja-183 variety, obtained at a dose of 300 Gy, was 3.55 t/ha, and the productivity of the same variety at a dose of 200 Gy was 3.6 t/ha. For the Ganja-182 variety, this parameter was 3.5 and 3.48 t/ha at doses of 5 and 100 Gy, respectively, and for the Ganja-160 variety, it was 3.56 t/ha at a dose of 100 Gy.

Table 5 Productivity of mutant forms and technological parameters of their fibers.

Mutants	The origin of mutants	Maturation period, days	Productivity		Fiber yield, %	Fiber productivity		Mass of a boll, (g)	Fiber length, mm	Fiber quality			
			t/ha	%		t/ha	%			Breaking load of fiber (g.g)	Linear density of fiber, m/tex	Relative breaking length, g.g/tex	Staple length, mm
Standard	Ganja-160	124	2.98	100	38.2	1.14	100	6.1	34.3	4.4	170 (5870)	25.8	33/34
MR-17	Ganja-160 - 100 Gy	122	3.56	119	40.1	1.43	125	5.5	34.6	4.2	170 (5875)	24.7	30/31
MR-21	Ganja-160 - 100 Gy	123	3.45	116	40.4	1.39	122	5.6	34.8	4.0	161 (6193)	24.8	30/31
MR-23	Ganja-160 - 100 Gy	122	3.30	110	40.1	1.32	116	5.8	33.8	4.0	153 (6515)	26.1	33/34
MR-30	Ganja-182 - 5 Gy	126	3.24	112	38.1	1.23	108	6.2	36.6	4.1	186 (5383)	22.1	31/32
MR-33	Ganja-182 - 5 Gy	124	3.40	114	39.5	1.34	118	5.8	35.0	4.7	187 (5332)	25.1	31/32
MR-35	Ganja-182 - 5 Gy	123	3.50	117	39.4	1.38	121	6.0	35.4	4.6	179 (5592)	25.7	33/34
MR-36	Ganja-182 - 10 Gy	123	3.16	106	38.7	1.22	107	6.7	35.2	4.2	156 (6424)	27.0	32/33
MR-49	Ganja-182 - 100 Gy	125	3.05	102	38.1	1.16	102	5.5	36.1	3.1	187 (5359)	16.6	30.31
MR-50	Ganja-182 - 100 Gy	124	3.48	117	38.0	1.32	116	6.6	34.8	4.3	176 (5689)	24.5	34/35
MR-67	Ganja-183 - 50 Gy	124	3.39	114	38.5	1.31	115	5.5	35.9	4.0	164 (6106)	24.4	32/33
MR-73	Ganja-183 - 200 Gy	126	3.60	121	41.6	1.50	132	5.4	35.2	4.7	199 (5012)	23.6	30/31
MR-75	Ganja-183 - 300 Gy	127	3.55	119	38.1	1.35	118	5.1	33.9	4.1	182 (5489)	22.5	32/33
MR-77	Ganja-183 - 300 Gy	126	3.30	111	41.5	1.37	120	5.8	33.8	4.5	203 (4921)	22.1	31/32
MR-99	Ganja-160 - 50 Gy	123	3.26	109	39.6	1.29	113	6.0	34.6	4.2	170 (5886)	24.7	32/33
MR-101	Ganja-160 - 100 Gy	122	3.37	113	38.9	1.31	115	6.8	34.8	4.3	174 (5741)	24.7	31/32
MR-106	Ganja-160 - 200 Gy	124	3.30	111	38.3	1.26	111	5.8	34.1	3.6	184 (5449)	19.6	32/33
MR-107	Ganja-160 - 200 Gy	123	3.28	110	41.3	1.35	118	6.5	34.3	4.3	210 (4757)	20.5	32/33
MR-108	Ganja-160 - 200 Gy	125	3.35	112	40.0	1.34	118	6.0	34.1	4.5	190 (5261)	23.7	33/34

We would like to note that the non-irradiated Ganja-160 cotton variety was adopted as the standard, yielding 29.8 t/ha. As can be seen, the mutant forms we selected had yields 15% higher, and in some cases, even 20% higher yield than the standard.

Significant differences in the yield and fiber productivity of the mutant forms were also observed. While the fiber yield of the standard form was 38.2%, those for the mutants Ganja-160 (at 100 Gy), Ganja-160 (at 200 Gy), Ganja-183 (at 200 Gy), Ganja-183 (at 300 Gy), and Ganja-182 (at 5 Gy) were 40.4, 40.0, 41.6, 41.5, and 39.5%, respectively. In other words, the results show that irradiation increases fiber yield by 2-4%.

Clearly, an increase in fiber yield should also be reflected in its productivity. Our results indeed show a 25-30% increase in fiber productivity for some mutants. For example, fiber productivity was 25% higher for the Ganja-160 (100 Gy) mutant and 32% higher for the Ganja-183 (200 Gy) mutant.

Radioactive irradiation also affected fiber length at some doses. Our fiber length results showed that the fiber lengths for the mutant forms Ganja-182 (5 Gy), Ganja-183 (100 Gy), and Ganja-183 (50 Gy) were 36.6, 36.1, and 35.9 mm, respectively. The fiber length of the standard grade, however, was 34.3 mm.

Considering that improving technological parameters such as fiber linear density, relative breaking load, and staple length increases cotton's competitiveness, these traits were also identified in some of the mutants. It was clear that in some variants, certain changes could occur in these parameters. For example, while the breaking load of the fiber, which determines fiber strength, was 4.4 g/g for the standard sample, this parameter was 4.7 g/g for the Ganja-182 (10 Gy) and Ganja-183 (200 Gy) mutant forms. The fiber linear density was 170 (5870) m/tex for the standard, and 186 (5383), 187 (5359) and 199 (5012) m/tex for the Ganja-182 (5 Gy), Ganja-182 (100 Gy) and Ganja-183 (200 Gy) mutants, respectively. A small increase in the relative breaking length was observed only in one variant. Thus, for the standard, this parameter was 25.8 g.g/tex, and for Ganja-182 (10 Gy), it was 27 g.g/tex. Our results for staple length showed no significant differences.

4. Discussion

It should be noted that there are many studies devoted to examining the variations caused by γ-irradiation of seeds before sowing in subsequent generations of cotton plants. These studies have shown that γ-irradiation of seeds enabled the production of cotton forms in the M5 generation that were superior to the control sample in terms of fiber yield, fiber length, and fiber strength [31]. An increase in the number of bolls per bush has been reported in a similar study [32].

The effect of gamma radiation on some agronomic characteristics such as plant height, number of branches per plant, number of bolls per plant, fiber fineness and cotton yield was also studied by Khan et al., [12]. The analysis of the results revealed significant differences in these parameters. Specifically, gamma radiation was found to have a positive effect on these parameters at low doses, whereas high doses had a negative effect.

In the work of Yilmaz et al., [33], 17 mutant lines of Acalpi-1952 and Sayar-314 cotton varieties were obtained in the M4 generation of irradiated doses of 100, 200, 300 and 400 Gy before sowing seeds. In the M5 generation, agricultural and technological characteristics such as the height of mutant plants, the number of monopodial and sympodial branches, the number of bolls, mass of one boll, yield, length, strength and fineness of fiber were studied. Depending on the irradiation dose, positive and negative changes were recorded in the mentioned parameters compared to the control.

The positive and negative effects of radioactive treatment of seeds on the number of plant branches and bolls were also demonstrated by Muhammad et al., [34].

The ability of gamma radiation to produce different effects at different doses has also been established for other plants. For example, El-Khateeb et al., [35] showed that all gamma irradiation doses had significant impacts on vegetative growth, i.e., plant height, number of branches per plant, compared with the control. The authors reported that doses of 5 and 10 Gy shortened the flowering period. However, these doses increased the number of flowers to a maximum for M1 and M2 generations, respectively. The mutants in this study were obtained from plants treated with doses of 15, 20, and 25 Gy.

In another study, a mutant NIAB-852 cotton variety was obtained via pollen irradiation, and also exhibited high yield and quality indicators [36]. The mutant NIAB-852 displayed desirable fiber quality traits, i.e., ginning out-turn percentage 38.8%, fiber length 30.1 mm (long-staple category), fiber fineness 4.68 μg/inch, uniformity index 83.1%, fiber maturity 81.1% and fiber strength 94.3 TPPSI. The authors concluded that the method of low-dose irradiation of cotton pollen, which effectively stimulates this process, improves the agronomic characteristics and disease resistance of plants [36].

Aslam et al., [20] also conducted research to create a new gene pool and select new cotton mutants with desired traits. The authors induced mutations using gamma irradiation. The selected mutants were evaluated for higher yield and yield contributing traits in different generations (M3-M6). Significant differences compared to parents were observed in the evaluated mutated generations. Furthermore, the mutant's raw cotton yield was higher than that of standard varieties. The mutant also possessed desirable fiber quality traits, such as fiber yield, fiber length, and fiber fineness.

5. Conclusion

Gamma-irradiation of seeds of the Ganja-160, Ganja-182, and Ganja-183 varieties, obtained by self-pollination, did not only result in abundant phenotypic changes, high genetic diversity, and a wide range of mutations in subsequent generations, but also enabled the production of mutant forms with 4-5, and in some cases, even 6, economically important traits (productivity, high fiber yield, long and strong fiber, etc.) for breeding.

Summarizing the obtained results, it can be concluded that:

- It was possible to select 113 mutant forms from the M4 generation plants that retained the traits of the previous M1, M2, and M3 generations, such as large bolls, compact bushes, high productivity, early maturing, high fiber yield, tall or short plants, wilt resistance, etc.;
- Increasing the gamma radiation dose increase the number of mutated families. Plants of the M4 generation had a wide range of mutations with 4-5, or even 6, economically important traits;
- At doses of 200 and 300 Gy, the Ganja-183 variety, and at doses of 100 Gy, both the Ganja-182 and Ganja-160 varieties demonstrated higher yields compared to the standard variety;
- An increase in fiber productivity of 25% and 32% was recorded for the Ganja-160 (100 Gy) and Ganja-183 (200 Gy) mutants, respectively;

- The fiber length of the standard variety was 34.3 mm, and the length of the fiber of the mutant forms Ganja-182 (5 Gy), Ganja-183 (100 Gy) and Ganja-183 (50 Gy) was 36.6, 36.1 and 35.9 mm, respectively;
- The breaking load of the fiber, which determines its strength, was 4.4 g/g for the standard sample, while this parameter was within 4.7 g/g for the mutant forms Ganja-182 (10 Gy) and Ganja-183 (200 Gy);
- Another quality indicator, fiber linear density, was 170 (5870) m/tex for the standard, while for the mutants Ganja-182 (5 Gy), Ganja-182 (100 Gy) and Ganja-183 (200 Gy), this indicator was 186 (5383), 187 (5359) and 199 (5012) m/tex, respectively.

These results demonstrate that radiation mutagenesis is an effective and feasible method for creating mutant plant libraries.

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Author Contributions

Zeynalova İ., Jafarov A., Allahverdiyeva L., Huseynova S., Eminova N. - Participated in the cultivation of cotton, conducting periodic phenological observations on it, identifying transformed plants, harvesting raw cotton at the end of the vegetation period, determining the quality and quantity of fiber. Jafarov E., Tagiyev A., Velijanova M., Gojayeva G., - Participated in conducting experiments on the determination of quantity and quality indicators, calculating statistical analyses, and writing the article. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

Data Availability Statement

All data generated or analyzed during this study are included in this manuscript.

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