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ANALYSIS OF THE INHERITANCE OF CHANGES INDUCED BY GAMMA IRRADIATION IN THE M_2 GENERATION OF COTTON VARIETIES GANJA-160 AND BA-440 TO THE NEXT M_3 GENERATION

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Abstract: The study explores the radiation effects on various generations of Ganja-160 and BA-440 cotton varieties subjected to diverse doses of γ-rays using the Co^{60} isotope prior to seed sowing. Phenological observations were conducted on both M_1 and M_2 generations of plants, spanning from germination to full growth, to identify the variations. The investigation delves into whether the observed variations in M_1 are of hereditary or modification origin.

It was found that high doses of γ-irradiation disrupted the germ cell of the seed, resulting in a broad range of genetic variation in plants and creating extensive opportunities for the selection of individuals with numerous valuable traits.

Furthermore, with an increase in the dose of gamma radiation, the number of mutated families increased. In the high-radiation variants, bushes with a large fiber yield, large cotton balls, and compact shapes were obtained. Some variants produced plants with 5-6 or even 7 economically valuable traits (large cotton balls, compact bush, productive, fast-growing, high fiber yield, and tall, short, wilt-resistant, etc.) and exhibited a diverse mutation spectrum.

Keywords: Ganja-160 and BA-440 cotton varieties, radiation mutagenesis, M_2 and M_3 generation plants, modification, and genetic variation.

1. Introduction

To ensure the dynamic development of raw cotton production in our country and to meet the growing demand of the textile industry for raw cotton, there is a significant need for the development of new cotton varieties that are productive, fast-growing, resistant to diseases and pests, and possess high fiber quality. In this regard, the creation of new cotton varieties, enabling an intensive increase in cotton productivity and their implementation in farms, is not only a necessary practical concern but also an urgent matter of great scientific importance.

As known, one of the most effective methods for obtaining economically valuable starting material in cotton farming is experimental mutagenesis. In genetics and breeding studies, experimental mutagenesis is employed to accelerate breeding. This methodological approach is in high demand for creating cotton cultivars with desirable characteristics, as many problems in mutation-selection remain unsolved [1].

Currently, radiation technologies find widespread use across various sectors of the national economy, including agriculture. This is due to their advantages, such as the simplicity of application, low cost, environmental friendliness, stimulating effects in small doses, a high degree of neutralization of planting material with the absence of lethality, minimized damage to seeds during processing, absence of induced radiation, and reduced energy consumption [2].

Our research aims to acquire mutant cotton lines resistant to various diseases and extreme environmental factors by exploiting genetic variation induced in seeds through γ-rays, essentially utilizing radiation mutagenesis. These mutant lines are intended to serve as novel starting material for breeding purposes.

2. Experimental Part

Cotton varieties (Ganja - 160 and BA-440), obtained through two years of self-pollination and regionalized by the Agrarian Services Agency, were the subjects of our research.

The Ganja-160 variety was regionalized in 2016 and is recognized for its increased productivity, drought resistance, and high fiber quality, meeting the demands of the textile industry. This is crucial for the production of high-quality fabric. It has a shorter vegetation period compared to foreign varieties (120 days). Harvesting starts at the end of August and concludes in the first ten days of October.

The BA-440 variety, developed by Turkey's "Progen TOHUM AS" company, falls into the category of mid-late maturing cotton varieties. It features a compact bush, branched stem, oval cotton ball, and a yield of 45-50 sen/ha. With a vegetation period of 145-150 days, this variety boasts a high (44.4%) fiber yield and excellent fiber quality.

At the initial stage (for the M_1 generation of plants), dry seeds of both cotton varieties underwent γ-ray treatment at doses of 100, 200, 300, and 400 Gy using the Co^{60} isotope. Non-irradiated seeds of these varieties served as the control variant. The seeds corresponding to both the irradiated and control variants were planted in the experimental base of the Scientific Research Institute of Plant Protection and Technical Crops under open field conditions in the second ten days of April, according to the scheme of 90 x 10 cm (with 2 seeds in each nest, totaling 100 seeds for each variant).

Upon conclusion of the growing season, seeds from both modified and unmodified plants in M_1 were separately collected, stored under special conditions, and sown as families in the subsequent planting (for the M_2 generation of plants). In the second year of the study, that is, in the M_2 generation of plants, the same type of modified plants were separately collected according to the variants, and the seeds were used to cultivate M_3 generation plants. It was determined whether the changes detected in M_3 were genetic.

3. Obtained Results and Their Explanation

Based on the fact that gamma-irradiation is a mutagenic factor at high doses, research was conducted to obtain cotton genotypes resistant to adverse conditions and various diseases. At the initial stage, before sowing, seeds of plants treated with different doses of γ-rays were cultivated, and the characteristics of the growing plants were examined to identify those with altered traits. Considering that changes in the vegetation period, main stem height, number of sympodial branches, and number of cotton balls per bush are key criteria determining the mutation's effectiveness, these parameters, along with the main economic characteristics and quality indicators of cotton, were assessed.

In total, the raw cotton of 850 plants was harvested by individual sampling, and economic value indicators such as fiber yield, fiber length, fiber strength, raw cotton mass per cotton ball, etc. were determined. Following cotton harvesting, the modified and unmodified plants in M_1 were segregated through individual selection. Their seeds were collected separately, stored under special conditions, and utilized for sowing as a family in the subsequent planting, i.e., in M_2. To ascertain whether the changes observed in M_1 are genetic (mutagenic) or simply modification variation, the study extended not only to the M_2 generation of plants but also to the subsequent M_3 generation.

The results revealed that, in some cases, the altered traits of the same form were morphogenetic and not inherited by the next generation. It is noteworthy that the same altered trait can be both mutational and modificational. For instance, observations indicated that the change in the shape of the cotton ball was modified in the Ganja-160 variety at a radiation dose of 150 Gy, while in the BA-440 variety at the 300 Gy radiation dose, it was mutational.

Literature suggests that mutagens are more effective in inducing genetic variation for economically valuable traits in low doses than in medium and high doses [3].

It is commonly understood that γ-irradiation disrupts the seed germ cell, leading the plant organism to grow and develop by sprouting from both healthy and damaged cells. Therefore, for the normal development of mutant cells, plants should be cultivated with high agrotechnical standards, enhancing mutation yield and improving plant vitality [4].

It has long been recognized that the impact of gamma radiation at high doses halts the development of the main body of plants, while at low doses, it paradoxically stimulates the development processes [5].

Our research on the morphophysiological changes induced by gamma-irradiation in the studied plants revealed that the irradiation produced more sterile and polysterile plants in the M_1 generation of Ganja-160 and BA-440 varieties. Irradiation of seeds with high doses leads to an increase in chlorophyll mutations, resulting in plants characterized by the absence of chlorophyll in the leaves. In plants with chlorophyll mutations, once 3-4 green leaves were formed, the others were destroyed. The height and development phases were normal in all variants during the early stages of vegetation in modified plants, but later, significant differences emerged. Despite the formation of normal cotton balls during reproductive development in these plants, most remained unopened by the end of the vegetation period, and mature cotton balls were predominantly located in the lower part of the sympodial branches of the plants.

Observations indicated that the impact of γ-irradiation on seeds before sowing led to various types of changes in the researched plants, with the number of modified plants varying in both cotton varieties based on the radiation dose. Generally, no consistent patterns in variety variability were discerned, and the variations appeared to be random. Nevertheless, in some cases, changes induced by γ-irradiation were found to occur in a similar manner.

As mentioned earlier, seeds from both modified and unmodified plants in M_1 were individually collected and sown in the subsequent step. All plants were also individually studied in M_2.

The study of the variability of M_2 generation plants revealed that traits such as late-maturing, twining of branches, small cotton balls, etc., observed in M_1 were modification [6].

Given that γ-irradiation can induce a broad spectrum of genetic variation, creating ample opportunities for selecting individuals with valuable traits [7]. Taking this fact as a basis, phenological observations were conducted in both M_1 and M_2 generations. These observations spanned from the germination phase to the full growth phase to identify changes in the plants, and it was investigated whether the changes in M_1 were of hereditary or modification nature. To achieve this, a comprehensive analysis of the changes occurring in M_1 under the influence of γ-irradiation was conducted, and the obtained results are presented in Table 1.

Table 1

Indicators of the type of change occurring in M_1 under the influence of γ-irradiation

Variants	Radiation dose, Gy.	Quantity of modified plants, number	Type and quantity of variation in plants, number											
			Shape of bush						Shape of cotton ball					
			Compact	Scattered	Strongly branched stem	Fascination on branches	Dwarf	Clustered conifers	Large	Small	Late-maturing	Sterile	Semi-sterile	Early-maturing
Ganja-160														
1	Control	-												
2	100	2	1											1
3	200	25	5	2	1	2	1	2	1	3	2		3	3
4	300	28	4	4	3	3	2	3	3		3	2	1	
5	400	33	4	5	3	6	2	2	4		3	3	1	
BA-440														
6	Control	-												
7	100	27	7	2		3	1		2	3	2	2	3	2
8	200	29	3	6	4	2	2	3	3		3	1	2	
9	300	28	3	4	5	2	2	3	4		2	2	1	
10	400	25	1	4	3	3	1	2	3		3	4	1	

The indicators of the number of modified plants formed in M_1 are given in Table 2.

Table 2

Indicators of the number of changes in M_1 due to the γ-irradiation

No.	Radiation dose, Gy	The number of plants counted in M_1, in numbers	Number of modified plants, in numbers	Number of modified plants, in %
Ganja-160				
	Control	394	-	-
2	100	314	2	0.63±0.4
3	200	281	25	8.89±1.7
4	300	270	28	10.37±1.8
5	400	210	33	15.71±2.5
BA-440				
1	Control	378	-	-
2	100	302	27	8.94±1.6
3	200	232	29	12.50±2.2
4	300	199	28	14.07±2.5
5	400	147	25	17.00±3.1

In the subsequent phase, the yield from modified plants in M_1 was collected through individual selection, and sown as a family in M_2. Additionally, seeds obtained from plants that did not differ from the control variant (potentially having a recessive mutation) were also sown as a family.

Considering the potential emergence of new mutations in the M_2 and M_3 generations of plants, families altered in M_2 were sown in M_3 and the genetic nature of the M_3 generation was examined.

In line with several studies where families altered in the M_2 generation were chosen based on the selection method and recognized as mutant forms [8, 9], we scrutinized materials obtained in the M_2 and M_3 generations, studying the mutation yield and frequency. The research work undertaken was not only challenging but also demanded a large area. Visual field inspections and laboratory analyses were conducted at the end of the vegetation period to identify modified forms. Since the primary objective is to identify forms with economically valuable traits and select forms with complex characteristics, the harvest was gathered through individual selection. Even if the mutant form is multi-rowed, the selection material is not collected in groups and families. The primary reason for collecting the crop of the mutant cotton form through individual selection is also explained by the highly heterozygous nature of the genetic material, reflected in the phenotype.

The effectiveness of the experimental mutagenesis method is known to depend significantly on the amount of material used in the experiment. Therefore, the greater the number of plants in M_1, the more diverse and numerous the modified plants. In such cases, there is a high probability of obtaining mutant forms with economic value. The genotype of varieties is also crucial in this process, as similar mutations can occur in genotypes close to each other [10].

In the M_2 generation of plants, the study of morphological changes caused by γ-irradiation in all variants of plants was continued. It became evident that, unlike the forms in M_1, the forms selected for branching type, ball size, fiber yield and length, productivity, shortening of the vegetation period, height, and hairiness of the main stem are more hereditary.

Here is a brief description of some of the most common modified plants selected from cotton varieties:

- Plants with a changed ball shape. This type of variation is frequently observed, resulting in not only large cotton balls but also small and elongated ones. Most deformed cotton balls are formed at high values of radiation dose.
- Plants with a changed branching type. Numerous compact and scattered types of plants are found at large values of gamma radiation (300 and 400 Gy doses).
- Deformed plants. The plants selected for this feature differed from the initial varieties, particularly in terms of the mass of raw cotton per bush. These distinctions were observed at radiation doses of 10 Gy and higher. The plants chosen in M_2 were superior to the control plants in terms of the number and size of cotton balls on the bush.
- Plants with a shortened vegetation period. In M_2, a substantial number of plants with a shortened vegetation period due to the radiation effect were obtained. Early maturing forms were selected from initial cultivars [11].
- Plants that changed color. This group encompasses plants that altered the color of their leaves, main stems, and cotton balls. This may include plants with varying degrees of anthocyanin spots on generative and vegetative organs.
- Hairiness. The plants chosen for this feature differ from the initial varieties in that their leaves and stems exhibit pronounced hairiness.
- Sterile plants. These are plants unable to fertilize and produce seeds. Sterile plants, resulting from the effect of gamma-irradiation, may also exhibit chlorophyll mutation in

the leaves. The chlorophyll mutation characterizes the effectiveness of the dose of γ-irradiation and the mutability of varieties.

From the findings of our study, it became evident that the increase in the dose of γ-irradiation leads to an escalation in the frequency of chlorophyll mutation in the M_2 generation of both studied plants, the number of mutant families, and concurrently, the mutation frequency in M_3.

The changes in the M_2 generation of plants and the summarized results of the inheritance of these changes to the next M_3 generation are reflected in Table 3.

Table 3

Indicators of the inheritance of changes in M_2 to M_3

No.	Gamma radiation dose, Gy	Number of families studied, number	Modified families in M_2		Plants inherited to M_3	
			Quantity, Number,	%, $(x \pm S_x)$	Quantity, Number	%, $(x \pm S_x)$
Ganja-160						
1	Control	394	-	-		
2	100	314	1	0.32 ± 0.3	1	0.32 ± 0.3
3	200	281	14	5.0 ± 1.3	6	2.22 ± 0.8
4	300	270	17	6.3 ± 1.4	8	3.0 ± 0.3
5	400	210	20	9.5 ± 0.1	7	3.4 ± 1.2
BA-440						
1	Control	378	-	-		
2	100	302	12	4.0 ± 1.1	4	1.4 ± 0.6
3	200	232	18	7.8 ± 1.7	6	2.6 ± 1.0
4	300	199	15	7.5 ± 1.9	6	3.0 ± 1.2
5	400	147	14	9.5 ± 2.4	4	2.7 ± 1.3

From the presented table results, it is evident that only 1 family (0.32 ± 0.3%) underwent changes in the M_2 generation of Ganja-160 cotton variety under a γ-irradiation dose of 100 Gy and this family was inherited to M_3. At a γ-radiation dose of 200 Gy, 14 families (5.0 ± 1.3 %) experienced changes in M_2, with 6 families (2.22 ± 0.8) inheriting M_3. For the radiation dose of 300 Gy, 17 families (6.3 ± 1.4%) out of 270 studied altered in M_2, with 8 families (3.0 ± 0.3 %) inheriting M_3. At a radiation dose of 400 Gy, out of 210 studied families, 20 families (9.5 ± 2.4 %) were mutated in M_2, and 7 families (3.4 ± 1.2 %) were inherited to M_3.

Different outcomes were obtained for the BA-440 cotton variety. At an irradiation dose of 100 Gy, 12 families (7.8 ± 1.7 %) out of 302 studied families changed in M_2, and 4 of them (1.4 ± 0.6 %) were inherited to M_3. For the radiation dose of 200 Gy, 18 families (7.8 ± 1.7%) out of 232 studied families underwent changes in M_2, and 6 families (2.6 ± 1.0%) out of 18 modified families (7.8 ± 1.7%) were inherited to M_3. In the case of the radiation dose of 300 Gy, 15 families (7.5±0.9 %) were altered in M_2 out of 199 studied families, and 6 families out of altered families (3.0±1.2 %) inherited M_3. At a radiation dose of 400Gy, out of 147 studied families, 14 families (9.5 ± 2.4%) altered in M_2, and 4 families were inherited to M_3.

Summarizing our results, it can be stated that the increase in the radiation dose during the irradiation of cotton seeds leads to an increase in the number of mutated families. Exposure of seeds to radiation in high doses also causes an increase in cotton fiber yield, growth of cotton balls, and formation of compact bushes. Radioactive radiation has a wide spectrum of mutations, creating plants with 5-6, even 7 economically valuable traits (large cotton balls, compact bush, productive, fast-growing, high fiber yield, and tall, short, wilt-resistant, etc.) in some variants.

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АНАЛИЗ ПЕРЕДАЧИ ИЗМЕНЕНИЙ В ПОКОЛЕНИИ M_2 СОРТОВ ХЛОПКА ГЯНДЖА-160 И ВА-440, ВЫЗВАННЫХ ГАММА-ОБЛУЧЕНИЕМ, НА ПОСЛЕДУЮЩЕЕ M_3 ПОКОЛЕНИЕ

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Резюме: В представленной работе представлены результаты исследований радиационных эффектов на разных поколениях сортов хлопчатника Гянджа-160 и ВА-440, семена которых с помощью изотопа Со-60 перед посевом обработаны разными дозами γ-лучей семян. С целью выявления изменчивости проведены фенологические наблюдения как за M_1, так и за M_2 поколениями растений от фазы всходов до фазы полной зрелости. Проанализировано, имеют ли изменения, происходящие в M_1, наследственный или модификационный характер.

Установлено, что высокие дозы γ-облучения разрушают зародышевую клетку семени, что приводит к широкому спектру генетической изменчивости растений и большим возможностям для отбора особей со многими ценными признаками.

Большие дозы γ-облучения, помимо увеличения количество мутировавших семейств, также дали кусты с большим выходом волокон, крупными шишками и компактной формой. В некоторых вариантах были получены растения с 5-6 и даже 7 хозяйственно-ценными признаками (большой хлопковый кокон, компактный куст, урожайный, быстрорастущий, с высоким выходом волокна, высокорослый, низкорослый, устойчив к увяданию и т. д.) и имели широкий спектр мутаций.

Ключевые слова: сорта хлопчатника Гянджа-160 и ВА-440, радиационный мутагенез, M_2 и M_3 поколение растений, модификация и генетическая изменчивость.

QAMMA ŞÜALANMANIN GƏNCƏ-160 VƏ BA-440 PAMBIQ SORTLARININ M_2 NƏSLİNDƏ YARATDIĞI DƏYİŞKƏNLİKLƏRİN SONRAKI M_3 NƏSLİNƏ İRSİ KEÇMƏSİNİN TƏHLİLİ

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Xülasə: Təqdim olunan tədqiqat işində Co^{60} izotopunun köməyi ilə toxumları səpindən əvvəl müxtəlif dozalarında γ-şüalarla işlənmiş Gəncə-160 və BA-440 pambıq sortlarının müxtəlif nəsillərində radiasiya effektləri araşdırılmışdır. Əmələ gəlmiş dəyişkənliyi aşkar etmək üçün bitkilərin həm M_1, həm də M_2 nəsilləri üzərində cücərmə fazasından tam yetişmə fazasına kimi fenoloji müşahidələr aparılmış, M_1-də olan dəyişkənliklərin irsi və yaxud modifikasiya xarakterli olması öyrənilmişdir.

Müəyyən edilmişdir ki, yüksək dozalarda γ-şüalanma toxumun rüşeym hüceyrəsini pozmuş, nəticədə bitkilərdə geniş diapazonlu irsi dəyişkənlik baş vermiş və bir çox qiymətli əlamətlərə malik fərdlərin seçilməsi üçün geniş imkanlar yaranmışdır.

Qamma-şüalanma dozasının artması mutasiyaya uğramış ailələrin sayının artmasına səbəb olmaqla yanaşı, yüksək şüalanma variantlarında həm də böyük lif çıxımlı, iri qozalı, yığcam formalı kollar alınmışdır. Bəzi variantlarda 5-6, hətta 7 təsərrüfat qiymətli əlamətləri (iriqozalı, yığcam kol, məhsuldar, tezyetişən, yüksək lif çıxımlı və uzunboylu, qısaboylu, viltədavamlı və s.) olan bitkilər alınmaqla, geniş mutasiya spektrinə malik olmuşlar.

Açar sözlər: Gəncə-160 və BA-440 pambıq sortları, radiasiya mutagenezi, M_2 və M_3 nəsil bitkilər, modifikasiya və irsi dəyişkənliklər.