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OBTAINING MUTANT COTTON FORMS ON THE BASIS OF RADIATION TECHNOLOGY

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Abstract: The work is devoted to the study of mutations and modifications caused by radioactive radiation in the M_1 generation of Ganja-160, Ganja-182, and Ganja-183 cotton varieties treated with gamma rays in doses of 5, 10, 50, 100, 200, 300, and 400 Gy before sowing.

Results on the effect of gamma radiation treatment on seed germination and plant viability, as well as on the biomorphological characteristics of plants (vegetation duration, height, number of sympodial branches, number of cones per bush) have been obtained. At the end of the vegetation, altered plants were recorded in the M_1 generation, which differed from the original varieties in terms of phenotypic characteristics in each variant, and sterile, semi-sterile, fertile, and chlorophyll-free forms generated in the plants were identified. It turned out that gamma radiation at high doses can also affect the number (3, 4, or even 5 cones in a fruit organ) and the shape (main body hairy, oblong, ovate, sharp-nosed cones) of cones in the fruit organs.

In the end, the raw cotton of the modified (different from the original varieties in some respects) and unchanged plant samples were collected as individual samples. The aim was to sow these seeds as a family in M_2 and select a breeding material to obtain mutant forms that are resistant to adverse conditions and various diseases based on radiation technology.

Keywords: radiation mutagenesis, cotton varieties, breeding material

1. Introduction

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

As it is known, breeding methods based on artificial, ie induced mutations are preferred to obtain plant genotypes that are resistant to pests and various extreme environmental factors, as well as have better quality indicators [1; 2]. This method, called mutation breeding, is somewhat random, unlike mutations, which are obtained by genetic engineering and allow for purposeful genetic modification [3].

Up to date, various methods have been used for plant breeding by mutation. Until recent years, breeders have preferred to obtain sustainable forms of plants (hybrids, lines, and varieties) based on the use of chemical mutagens. For example, it has been possible to obtain a pest-resistant and fast-growing cotton variety based on chemical mutagenesis [4]. Although these methods give good results, they are not only unpleasant in some cases, such as the toxic properties of the used substances, but also labor-intensive and expensive (high cost) processes.

Today, the use of radiation technologies in all sectors of the economy, including agriculture, has become widespread. The main reason for the widespread use of radiation technology in recent years is its simplicity, low cost, being environmentally friendly technology, and having many advantages in small doses (having a stimulating effect, high degree of neutralization of planting material, lack of lethal end to planting material, minimizing seed damage during processing, absence of induced mutation, reducing energy consumption) [5; 6].

In agriculture, as is known, radioactive radiation is used for 3 main purposes. These include the low-dose irradiation of plants themselves or their seeds before sowing to stimulate the growth and development of plants, the use of radiation sterilization methods to protect agricultural crops from pests, and the use of ionizing radiation for radiation mutagenesis and plant breeding.

The latter direction is based on the fact that radioactive radiation is a mutagenic factor, and in this direction, it is possible to obtain plant genotypes that are resistant to adverse conditions and various diseases. For this purpose, plant sprouts or plant seeds before sowing are irradiated with gamma rays in relatively high doses, then the irradiated material is cultivated and new planting material, ie seeds or seedlings, is obtained from them. Then the characteristics of the growing plants are studied. Subsequent generations of plants with changed characteristics are also studied. As the application of gamma radiation accelerates the process of natural genetic mutation, the time required for this process is significantly reduced.

Based on radiation mutagenesis, it was possible to obtain a mutant corn line, which was resistant to diseases such as blister smut, fusarium wilt, white rot, and this group of diseases [7].

Research has also been conducted to study the joint effect of gamma radiation with chemical mutagens. For example, to obtain genetic diversity, it has been used joint treatment of cotton seeds and pollen with gamma radiation and chemical mutagens before intra-varietal and inter-varietal cross-linking and it was possible to obtain a morphologically stable line that is productive, resistant to wilt disease, and has high economic value [8].

Today, there is a great need for scientific research to ensure the dynamic development of raw cotton production in our country, to stimulate the creation of new varieties of cotton that are productive, fast-growing, disease and pest resistant, and high fiber quality for meeting the needs of the textile industry. It is no secret that the pace of development of society requires the development of the cotton industry, which is a valuable raw material not only for the textile industry but also for various sectors of the economy in general. From this point of view, it is necessary to create new high-potency cotton varieties and apply them to farms as a progressive method that can ensure intensive growth of cotton productivity.

It is clear from the results of research conducted so far that one of the most effective ways to obtain valuable farm raw material in cotton is experimental mutagenesis. Experimental mutagenesis, which is widely used in genetics and breeding research, is known to be used to intensify breeding work [9, 10]. Studies show that there are few new methodological approaches to the creation of cotton varieties with the desired characteristics by experimental mutagenesis, many problems of mutation breeding are still unresolved and there is a great need for them today.

Our research aims to create hereditary variability in cotton seeds under the influence of gamma rays, as well as a new starting material for breeding.

2. Materials and methods

As objects of research, it was used three cotton varieties (Ganja - 160, Ganja - 182, and Ganja - 183), were obtained from the self-pollination of seeds within 2 years and regionalized by the Agrarian Services Agency.

Ganja - 160 varieties were regionalized in 2016. This variety is more productive, drought-resistant, and has high fiber quality, ie high fiber quality that meets the needs of the textile industry, which is very important for the production of high-quality fabric. The vegetation period is 120 days. The harvest begins in late August and ends in the first decade of October. The vegetation period of foreign varieties lasts until December.

Ganja - 182 varieties were regionalized in 2018. This variety is the fastest-growing cotton variety. The vegetation period is 115 days. Compared to the Ganja-160, the body is compact, the cones are closer to the body and are very convenient for harvesting by machine.

Ganja - 183 variety is regionalized in the republic in 2020, and fiber yield is high (42%). It can compete with foreign varieties with a fiber yield of 42-43%. It is highly productive, branched, and has 2 types of branching.

All three varieties are suitable for local soil-climatic conditions, are fast-growing and productive

Before sowing, cotton seeds were treated with gamma rays in doses of 5, 10, 50, 100, 150, 200, 300, and 400 Gy with the help of Co-60 isotope in the RUXUND-20000 device in the Scientific-Experimental Complex "Isotope Radiation Sources" of the Institute of Radiation Problems of ANAS (capacity was 0.342 Rad/sec in all cases (Figure 1)).

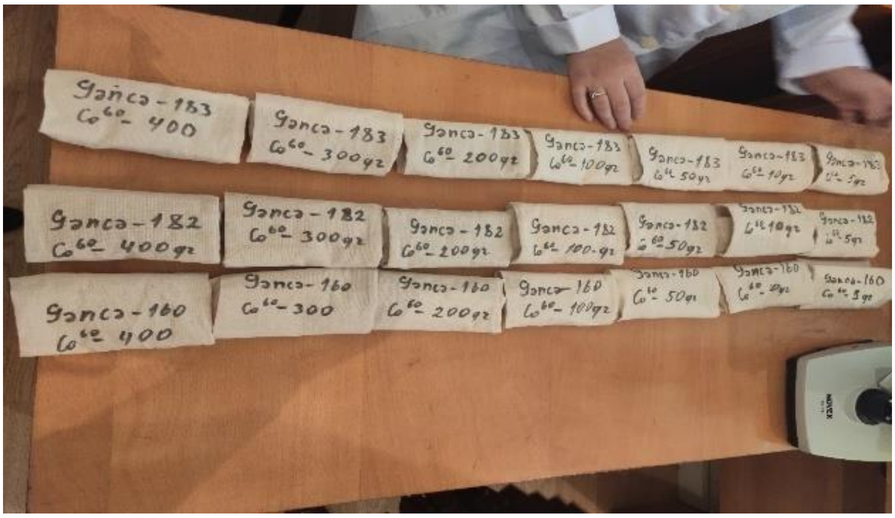

Fig. 1. Preparing seeds for irradiation

Non-irradiated seeds of these varieties were used as a control variant. Irradiated cotton seeds together with non-irradiated ones were sown in the experimental base of Ganja Regional Agrarian Science and Innovation Center in open field conditions in the second decade of April according to the 90x10 cm scheme, in 4 repetitions with 100 seeds in each variant. Considering that experimental mutagenesis did not allow dispersion, a limited number of seeds (2) were sown in each nest. As is well known, dispersion in the field can lead to a violation of the mutation rate and the destruction of important, positively altered plants.

At the end of the vegetation, the raw cotton of 20 cones from the 1st and 2nd places of the 2nd-5th sympodial branches of plants in each variant, as well as the raw cotton of all plants in the experimental field were collected as individual selection.

3. Results and their explanation

A few days after sowing, field germination of seeds was recorded.

The results obtained on the germination capacity of cotton seeds have been presented in Table 1.

Table 1

Indicators of the effect of gamma radiation treatment of seeds on their ability of field germination and plant viability

Variants	Radiation doses, Gy	Seed germination ability		The viability of plants	
		A number of seedlings, numb.	Seed germination percentage (x ± sx)	Number of plants, in numbers	Number of plants, in percent (x±sx)
Ganja-160					
1	Control	320	80 ±2.50	316	79 ±2.58
2	5	312	78 ±2.65	296	74 ±2.96
3	10	308	77 ±2.73	292	73 ±3.04
4	50	300	75 ±2.89	292	73 ±3.04
5	100	280	70 ±3.27	268	67 ±3.51
6	200	260	65 ±3.67	220	55 ±4.52
7	300	140	35 ±13.63	100	25 ±8.67
8	400	72	18 ±21.34	60	15 ±11.90
Ganja-182					
9	Control	364	91 ±1.57	356	89 ±1.76
10	5	364	91 ±1.57	352	88 ±1.85
11	10	348	87 ±1.93	336	84 ±2.78
12	50	308	77 ±2.73	292	73 ±3.04
13	100	312	78 ±2.65	288	72 ±3.12
14	200	308	77 ±2.73	272	68 ±3.43
15	300	120	30 ±7.64	104	26 ±8.43
16	400	76	19 ±10.32	52	13 ±12.93
Ganja-183					
17	Control	344	86 ±2.02	336	84 ±2.48
18	5	328	82 ±2.34	308	77 ±2.73
19	10	320	80 ±2.50	316	79 ±2.58
20	50	312	78 ±2.65	276	69 ±3.35
21	100	284	71 ±3.19	240	60 ±4.08
22	200	276	69 ±3.35	224	56 ±4.43
23	300	104	26 ±8.43	60	15 ±11.90
24	400	36	9 ±15.90	20	5 ±20.00

Note: Seeds were sown in 4 repetitions with 100 seeds in each variant

The results show that the treatment of seeds with gamma rays before sowing clearly affects their ability to germinate in the field. More precisely, an increase in the radiation dose has a negative effect on the ability of seeds to germinate.

In the following stages, the duration of the development stages of flowering and maturation of plants in the M_1 generation, as well as the rate of opening of the cones were determined. By measuring 25 plants in each variant, it has been studied the effect of gamma radiation on the growth and development of the cotton plant during the mass budding, flowering, and growth phases, and on the viability of the plants at the end of the vegetation.

It is known that one of the main biological characteristics of various plants, as well as cotton, is their viability. When seeds are treated with gamma rays, the viability of plants is the process of regenerating plants after cell damage caused by gamma rays. From our systematic observations of all phases, it became clear that during the growing season, seedlings, unlike control plants, appear irregularly in the experimental variants, and are also delayed by 3-5 days (Figure 2).

Fig. 2. View of cotton plants grown in the experimental field of the Department of Technical Plant Breeding.

It is clear that there are obvious differences in the viability of plants grown from irradiated seeds compared to control plants (Table 1). Thus, increasing the radiation dose has a negative impact on the viability of plants. At doses higher than 200 Gy, the effect of gamma radiation on both seed germination and plant viability for all three studied varieties was more dramatic. For example, at a radiation dose of 400 Gy, the germination of seeds and viability of plants are respectively 4.4 and 5.2 times less for Ganja-160 variety, 4.7 and 6.8 times less for Ganja-182 variety, and 9.5 and 16.8 times less for Ganja-183 variety.

In the variants suitable for high radiation doses, the number of damaged seedlings was also high, and the kernel leaves became noticeably thicker and deformed by changing their shape. Also, the emergence of true leaves was delayed, the root system was not able to form the plant properly, and at different times of the growing season, these seedlings disappeared. Some of the seedlings remained in the form of kernels for a long time. At high doses of gamma radiation, poorly formed plants were also observed (Figure 3).

Fig. 3. Diversity of plant formation

Changes in the duration of vegetation, the height of the main trunk, and the number of sympodial branches and cones in one bush are considered to be the main criteria in determining the effectiveness of mutagenic factors. The results of our research in this area have been presented in Table 2.

Table 2

Results on the effect of gamma radiation treatment of seeds on biomorphological parameters of plants.

Variants	**Radiation doses, Gy**		Vegetation period	Height of plants	Number of sympodial branches	The number of cones in a branch
1	Ganja -160	Control	122	114.6	13.4	24.48
2		5	121	120.3	16.9	22.3
3		10	121	117.6	19	20.2
4		50	120	113	12.56	17.52
5		100	123	130.8	14.4	14.1
6		200	125	130.2	13.28	13.84
7		300	128	117	15.4	10.72
8		400	127	134.4	13.48	14.12
9	Ganja -182	Control	117	114.6	13.96	19.36
10		5	120	104.8	10.4	15.12
11		10	119	109	12.72	18.48
12		50	118	115	14.8	21.76
13		100	120	108.2	12.64	20.36
14		200	121	109.1	14.7	15
15		300	124	103	13.64	14.2
16		400	125	103	15	13
17	Ganja-183	Control	120	112	12.8	21.48
18		5	119	107.4	10.52	16.12
19		10	120	110.1	12	19.6
20		50	118	103.4	9.72	16.68
21		100	122	106.6	11.68	23
22		200	121	114.6	13.24	20.4
23		300	124	100	13.4	13.9
24		400	126	105	12.25	19.8

The presented results suggest that the listed parameters are sensitive to the effects of high doses of radioactive radiation. More precisely, the effect of gamma rays at high doses (more than 200 G) causes a significant prolongation of the vegetation period of all three varieties of cotton. It can be considered that at these doses, radioactive radiation slows down the growth of plants by inhibiting their development.

The effect of gamma rays at high doses was also able to significantly affect the number of cones in a bush. Thus, at 300 and 400 Gy radiation doses, the number of cones in one bush was 12 pieces less for Ganja -160 variety, 7 less for Ganja -182 variety, and 5 less for Ganja -183 variety compared to the control ones.

It was not possible to find any regularity in the change in the height of the main body and the number of sympodial branches, which depends on the radiation dose.

Our phenological observations on cotton varieties showed that despite the normal development of all three varieties at low doses, the growth of plants is delayed at doses above 200 Gy, and they can not perform normally. At high doses, the plants also grew sparsely and a large number of side branches grew from the main trunk (Figure 4).

Systematic phenological observations were made throughout the vegetation period in the M_1 generation. At the end of the vegetation, in each variant, altered plants differing from the original varieties in terms of phenotypic characteristics were recorded, and sterile, fertile, etc. forms generated in the plants, have been identified. It was found that sterile and semi-sterile plants are more common in variants with high values of gamma radiation doses (Figure 5).

Plants at high doses are also characterized by a lack of chlorophyll. In addition, the leaves of the plants turn green until the 2-3rd main leaves are formed, and the others are destroyed.

We believe that the presence of sterile, semi-sterile, and chlorophyll-free plants among the modified plants in M_1 can be considered as the occurrence of variability.

Fig. 4. Branching diversity of plants

Fig. 5. Chlorophyll diversity of plants

Our observations showed that among the plants that changed in high radiation doses in M_1, there are also plants that are fast-growing, elongated, and oval-shaped, having multi-slice cones, 3, 4, and even 5 cones clustered in a fruit organ, with elongation of lateral branches, stopping the development of the main trunk, with a trunk weak and densely hairy, with a large flower base, with leaves deeply sliced and heart-shaped, with large and small cones, with a scattered bush, as well as with altered shape, shortened inter-joint distance (Figure 6).

Fig. 6. Shape of fruit organs

The effects of gamma radiation, especially at high doses, have also affected the number and shape of cones in the fruit organs.

Cones with the hairy, elongated, ovate, sharp-nosed main body were found at different radiation doses, especially at doses above 200 Gy (Figure 6, 7).

Fig. 7. Cones in different shapes

It should be noted that plants with compact, zero, first, and pyramidal branches, as well as fast-growing, high-yielding forms, are of particular interest for practical breeding.

In the experiment, biomorphological indicators such as vegetation period of all three cotton varieties, plant height, the number of cones, and sympodial branches in the bush were determined.

Plants that differed in some of the initial varieties were selected, and their biomorphological and economic characteristics were analyzed. After the raw cotton of the samples was collected, the modified and unchanged plants were collected as an individual sample. The goal is to collect the seeds of both modified and unchanged plants separately in M_1 and sow them as a family in M_2. In total, 850 pieces of raw cotton from the plant were collected by individual breeding and their economic indicators such as fiber yield, fiber length, fiber strength, the weight of raw cotton in one cone, etc. are systematized for analysis in the laboratory. To determine whether the observed changes were genetic (mutagenic) or simply modified, the seeds were taken from these plants and stored for further sowing. It is also planned to conduct research in the 2^{nd}, 3^{rd}, and 4^{th} generations of these plants and to study their resistance to various diseases and environmental stressors for the breeding of plants, ie mutant forms with different characteristics that can be passed on to subsequent generations. Depending on the characteristics of mutation breeding, stable mutants stored in subsequent generations with one or more breeding traits will be crossed with the initial varieties (or among themselves), ie hybridization will be carried out according to the accepted methodology. The breeding of more valuable forms will be made by studying the observation of a separate mutant trait or a set of positive traits in a hybrid generation. Selected mutant hybrids will be studied according to basic breeding and economic value.

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References

1. Ahloowalia B.S., Maluszynski M. & Nichterlein_K. Global impact of mutation-derived varieties // Euphytica. 2004. Vol. 135, p. 187–204.
2. Pathirana R. Plant mutation breeding in agriculture // CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. 2011. Vol. 6, Iss. 032, p. 1-20.
3. Kartel N. A., Makeeva E. N., Mezenko A. M. Genetics. Encyclopedic Dictionary. Minsk: Belarusian Science, 2011.566 c, p. 234-241 (in Russian).
4. Gurbanov F.H. Breeding and seed production of agricultural crops. Baku, 2011, 384 p, pp. 41-45 (in Azerbaijani).
5. Farkhad S.A. and Hosseini A. Effect of gamma irradiation on antioxidant potential, isoflavone aglycone and phytochemical content of soybean (Glycine max L. Merrill) cultivar Williams // Journal of Radio analytical and Nuclear Chemistry. 2020, V.324, p. 497–505.

6. Verma A.K., Reddy K.S., Dhansekar P. and Singh B. Effect of acute gamma radiation exposure on seed germination, survivability and seedling growth in cumin cv. Gujarat Cumin-4 // Int. J. Seed Spices, 2017, V. 7(1), p. 23-28.
7. Al Silvi Anis Abdullah. Influence of agricultural practices on the phytosanitary state of sowing corn for grain. Dissertation. k. b. N. Krasnodar, 1998, 138 p, pp.61-68 (in Russian).
8. Asadov Sh. I. Experimental mutagenesis and hybridization in the creation of new forms of medium fiber cotton. Abstract of Doctor of Agricultural Sciences. Moscow, 1994. 34 p, pp. 15-18 (in Russian).
9. Guliyev R.A. Selection of plants with the basics of genetics. Baku, 2003. 276 p., pp.57-63 (in Azerbaijani).
10. Seyidaliyev N.Y. Genetics, selection and seed production Baku, 2010, 262 p., pp.144-153 (in Azerbaijani).

ПОЛУЧЕНИЕ МУТАНТНЫХ ФОРМ ХЛОПКА НА ОСНОВЕ РАДИАЦИОННОЙ ТЕХНОЛОГИИ

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Резюме. Работа посвящена изучению мутационных и модификационных изменений, вызванных радиоактивным излучением в поколении М1 сортов хлопчатника Гянджа-160, Гянджа-182 и Гянджа-183, семена которых перед посевом были обработаны γ-лучами в дозах 5, 10, 50, 100, 200, 300 и 400 Гр.

Получены результаты по влиянию лучевой обработки на всхожесть семян и жизнеспособность растений, а также на биоморфологические показатели (длительность вегетации, высоту растений, количество симподиальных ветвей, количество шишек на одной ветви) растений. По окончании вегетации в семействе М1 регистрировали измененные растения, которые отличались от исходных сортов по фенотипическим признакам в каждом варианте, и выделяли сформировавшиеся у растений стерильные, полустерильные, фертильные и бесхлорофильные формы. Установлено, что гамма-излучение в больших дозах может влиять также на количество (3, 4 и даже 5 шишек в одном месте) и форму (волосатые, удлиненные, яйцевидные, с острым носом) шишек в производственных органах.

В конце вегетационного периода хлопок как модифицированных (в некоторых отношениях отличающихся от исходных сортов), так и неизмененных образцов растений был собран как индивидуальная проба. Цель состояла в том, что посеять эти семена в М2 как семейство и на основе радиационной технологии получить мутантных форм, устойчивых к неблагоприятным условиям и различным заболеваниям, с последующим подбором селекционного материала.

Ключевые слова: радиационный мутагенез, разные сорта хлопчатника, селекционный материал.

RADİASİYA TEXNOLOGİYASININ ƏSASINDA MUTANT PAMBIQ FORMALARININ ALINMASI

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Xülasə: Iş toxumları səpindən əvvəl 5, 10, 50, 100, 200, 300 və 400 Qr dozalarda qamma şüalarla işlənmiş Gəncə-160, Gəncə-182 və Gəncə-183 pambıq sortlarının M_1 nəslində radioaktiv şüalanmanın yaratdığı mutasiya və modifikasiya dəyişkənliklərinin öyrənilməsinə həsr olunmuşdur.

Qqamma şüalarla işlənmənin toxumların tarla cücərmə və bitkilərin həyatilik qabiliyyətlərinə, həmçinin də bitkilərin biomorfoloji göstəricilərinə (vegetasiya müddətinə, hündürlüyünə, simpodial budaqların sayına, bir kolda olan qozaların sayina) təsirinə təsrinə dair nəticələr alınmışdır. Vegetasiyanın sonunda M_1 nəslində hər variantda başlangıc sortlardan fenotipik əlamətlərinə görə fərqlənən, dəyişilmiş bitkilər qeydə alınmış və bitkilərdə əmələ gəlmiş steril, yarımsteril, fertil və xlorofilsiz formalar müəyyənləşdirilmişdir. Aydın olmuşdur ki, yüksək dozalarda qamma şüalanma bar orqanlarında olan qozaların sayına (3, 4, hətta 5 qoza bir yerdə olan bar orqanlı) və formasına (əsas gövdəsi tüklü, uzunsov, yumurtavari, buruncuğu iti olan qozalar) da təsir edə bilir.

Sonda dəyişilmiş (ilkin sortlardan hansısa əlamətlərinə görə fərqlənən) və dəyişilməmiş bitki nümunələrinin xam pambığı fərdi seçmə kimi yığılmışdır. Məqsəd bu toxumları M_2-də ailə kimi səpmək və radisiya texnologiyalarına əsaslanmaqla əlverişsiz şəraitlərə və müxtəlif xəstəliklərə davamlı olan mutant formaların alınması üçün seleksiya materialının seçilməsi olmuşdur.

Açar sözlər: radiasiya mutagenezi, pambıq sortları, seleksiya materialı.