

ISSN 2518-1629 (Online),  
ISSN 2224-5308 (Print)

ҚАЗАҚСТАН РЕСПУБЛИКАСЫ  
ҰЛТТЫҚ ҒЫЛЫМ АКАДЕМИЯСЫНЫҢ  
Өсімдіктердің биологиясы және биотехнологиясы институтының

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## ИЗВЕСТИЯ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК  
РЕСПУБЛИКИ КАЗАХСТАН  
Института биологии и биотехнологии растений

## NEWS

OF THE NATIONAL ACADEMY OF SCIENCES  
OF THE REPUBLIC OF KAZAKHSTAN  
of the Institute of Plant Biology and Biotechnology

**БИОЛОГИЯ ЖӘНЕ МЕДИЦИНА  
СЕРИЯСЫ**



**СЕРИЯ**

**БИОЛОГИЧЕСКАЯ И МЕДИЦИНСКАЯ**



**SERIES**

**OF BIOLOGICAL AND MEDICAL**

**1 (325)**

**ҚАҢТАР – АҚПАН 2018 ж.  
ЯНВАРЬ – ФЕВРАЛЬ 2018 г.  
JANUARY – FEBRUARY 2018**

**1963 ЖЫЛДЫҢ ҚАҢТАР АЙЫНАН ШЫҒА БАСТАҒАН  
ИЗДАЕТСЯ С ЯНВАРЯ 1963 ГОДА  
PUBLISHED SINCE JANUARY 1963**

**ЖЫЛЫНА 6 РЕТ ШЫҒАДЫ  
ВЫХОДИТ 6 РАЗ В ГОД  
PUBLISHED 6 TIMES A YEAR**

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ALMATY, NAS RK**

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«ҚР ҰҒА Хабарлары. Биология және медициналық сериясы».

**ISSN 2518-1629 (Online),**

**ISSN 2224-5308 (Print)**

Меншіктенуші: «Қазақстан Республикасының Ұлттық ғылым академиясы» РҚБ (Алматы қ.)

Қазақстан республикасының Мәдениет пен ақпарат министрлігінің Ақпарат және мұрағат комитетінде  
01.06.2006 ж. берілген №5546-Ж мерзімдік басылым тіркеуіне қойылу туралы куәлік

Мерзімділігі: жылына 6 рет.

Тиражы: 300 дана.

Редакцияның мекенжайы: 050010, Алматы қ., Шевченко көш., 28, 219 бөл., 220, тел.: 272-13-19, 272-13-18,  
[www.nauka-nanrk.kz/biological-medical.kz](http://www.nauka-nanrk.kz/biological-medical.kz)

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Типографияның мекенжайы: «Аруна» ЖК, Алматы қ., Муратбаева көш., 75.

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«Известия НАН РК. Серия биологическая и медицинская».

**ISSN 2518-1629 (Online),**

**ISSN 2224-5308 (Print)**

Собственник: РОО «Национальная академия наук Республики Казахстан» (г. Алматы)

Свидетельство о постановке на учет периодического печатного издания в Комитете информации и архивов  
Министерства культуры и информации Республики Казахстан №5546-Ж, выданное 01.06.2006 г.

Периодичность: 6 раз в год

Тираж: 300 экземпляров

Адрес редакции: 050010, г. Алматы, ул. Шевченко, 28, ком. 219, 220, тел. 272-13-19, 272-13-18,  
[www.nauka-nanrk.kz](http://www.nauka-nanrk.kz) / [biological-medical.kz](http://biological-medical.kz)

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Адрес типографии: ИП «Аруна», г. Алматы, ул. Муратбаева, 75

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**News of the National Academy of Sciences of the Republic of Kazakhstan. Series of biology and medicine.**

**ISSN 2518-1629 (Online),**

**ISSN 2224-5308 (Print)**

Owner: RPA "National Academy of Sciences of the Republic of Kazakhstan" (Almaty)

The certificate of registration of a periodic printed publication in the Committee of information and archives of the Ministry of culture and information of the Republic of Kazakhstan N 5546-Ж, issued 01.06.2006

Periodicity: 6 times a year

Circulation: 300 copies

Editorial address: 28, Shevchenko str., of. 219, 220, Almaty, 050010, tel. 272-13-19, 272-13-18,  
<http://nauka-nanrk.kz/> / [biological-medical.kz](http://biological-medical.kz)

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Address of printing house: ST "Aruna", 75, Muratbayev str, Almaty

## NEWS

OF THE NATIONAL ACADEMY OF SCIENCES OF THE REPUBLIC OF KAZAKHSTAN

SERIES OF BIOLOGICAL AND MEDICAL

ISSN 2224-5308

Volume 1, Number 325 (2018), 35 – 43

UDC 575.633.11

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## UTILIZING INDUCED MUTAGENESIS IN KAZAKHSTANI WHEAT BREEDING

**Abstract.** Mutagenic effect of physical factors and chemical substances (aziridine or ethylene imine, nitro-ethylurea, nitrosoethyleneurea) leads to increase of the spectrum of hereditary variability for breeding purposes, which however is not studied in full extent. Ecological study of anthropogenic factors action leading to disruption of certain links between chemical elements and their combinations, raise of heavy metals concentration in soil, facilitate examination of mutagenic and toxic properties of heavy metals. Increase in wheat yields by improving its genotype is one of the most urgent problems of agriculture and economy. At present, using traditional methods of selection and genetic studies, such as backcross selection, distant hybridization, and experimental mutagenesis, increased efficiency of obtaining genetically modified and improved forms of wheat [1-5]. Heavy metals are defined as metals having a density higher than 5 g/cm<sup>3</sup>. Of the total 90 naturally occurring elements divided into three classes by the degree of their threat, 53 are considered heavy metals and few are of biological importance. Accumulation of heavy metals such as cadmium (Cd) in the environment is now becoming a major cause of environmental pollution. Toxic metals can inactivate proteins, shifting metal cofactors, blocking active centers or causing allosteric changes. Besides, large number of those possesses ability of inducing mutagenic changes, tumors and causing macroscopic changes. Molecular mechanism of heavy metals toxicity is not completely understood. Cd is non-essential element that negatively affects plant growth and development, released into the environment by power stations, heating systems, metal working industries or urban traffic, which has high cumulative effect with almost no biodegradation. In plants it affects such processes as stomata opening, transpiration and photosynthesis, consequently chlorosis, leaf rolls and stunting are the main symptoms of Cd toxicity in plants accompanied by root browning, leaf red-brownish discoloration. It can also reduce the absorption of nitrate from root to shoot by inhibiting the nitrate reductase activity in shoots. The negative effect of Cd on plant growth was accompanied by an increase in dry to fresh mass ratio in all organs. Several researches have suggested that an oxidative stress could be involved in cadmium toxicity, by either inducing oxygen free radical production, or by decreasing enzymatic and non-enzymatic antioxidants [6-9]. On the other hand, the use of induced mutagenesis showed high efficiency in the production of forms with high yield, quality bakery, lodging resistance, modified plant height and resistance. Moreover, this paper is an attempt of summarizing results performed by our group in this direction.

**Key words:** breeding, chemical mutagenesis, isogenic substituted wheat lines.

**Introduction.** Plant breeding requires genetic variation of useful traits for crop improvements. Chemical (base analogs, alkylators, ICR-compounds), physical (gamma rays, X-rays, ion beam) or biological (viruses, bacteria) agents can induce sudden heritable changes occurring in the genetic information of an organism not caused by genetic segregation or genetic recombination. Mutation breeding involves the development of new varieties by generating and utilizing genetic variability through chemical and physical mutagenesis [4]. Qualitatively new forms, such as dwarf mutants in wheat and barley, ultra-fast mutants in barley, resistant to fungal diseases of forms of plants, high-leasing and highly productive mutants serving as progenitors of new high-yielding varieties are obtained by chemical mutagenesis [3, 5]. However, obtaining mutants and their study – is only the first stage of selection work.

It is possible to use hybridization in selection of mutations. More important is the use of mutants in hybridization to obtain positive transgressions. Preparation of mutants and their use for hybridization requires the study of genetic nature of emerging changes, which is crucial for the selection of effective mutagens with specific action, and to broaden and deepen understanding of the nature of wheat evolution. Mutants having complex morphological, physiological and biochemical changes affecting economically valuable properties can be further used to locate genes that determine the trait followed Intervarietal replacement of chromosomes [11]. Isogenic lines are convenient objects for many biological and agricultural experiments. Main advantage of these lines is high genetic similarity among themselves and with the control line, which allows estimating the contribution to the formation of crop marking characteristics and applying them as effective donor marker signs. One of the possibilities to create new varieties with economically valuable traits, and primarily in the direction of selection on productivity and disease resistance to wheat rust, and their improvement in economically valuable attributes is the method of hybridization. Interspecific hybridization in wheat breeding to leaf rust resistance requires use of *T. timopheevii*. In order to overcome hybrids sterility methods facilitating the gene transfer from distant wheat species have been recently developed [10]. Some of them are based on the methods of chromosome engineering, others on methods of genetic control of meiotic recombination, third on the methods of genetic engineering. However, to obtain mutants and study them – this is only the first stage of the selection work. More important is the using mutants in the hybridization to obtain positive transgressions. Hybridization gives possibility to better use of mutations in wheat breeding. Obtaining of mutants and using them for hybridization require the study of the genetic nature of appearing changes, which have great importance for the selection of effective and specific action of mutagens, and for extension and deepening of understanding the nature of wheat evolution. The aim of our work is the obtainment of mutants with agriculturally valuable traits, distant and interspecific wheat hybrids and their breeding analysis. We have found that the treatment of seeds with  $CdCl_2$  induces changes in wheat, which are expressed in the appearance of the first generation (M1) of powerful plant with productive breeding and valuable traits - elongated ears, larger grains, grain weight with the main spike, 1000 grain weight, etc. These plants have the characteristics of the initial variety, but on a number of quantitative traits superior control options. Signs of altered forms stably transferred to the M2 generation - M4. In this regard, it was of interest to study the effect of 0.01% aqueous solution  $CdCl_2$  the variability of quantitative indicators of internal anatomical structures of wheat.

**Materials and methods.** Spring soft wheat Kazakhstanskaya 126 variety (*Triticumaestivum* L. var. *ferrugineum* Al.), a series of its monosomic lines and such varieties as Nadezhda, Kazakhstanskaya 4 and Shagala served the objects of the current research. Kazakhstanskaya 126 variety was developed at Kazakh SRI of Agriculture and crop production by crossing soft wheat Lutescence 47 with the local variety Kozhebiday and subsequent two-time selection. Isogenic lines of Avocet variety by *Yr* genes, *T. timopheevii* species. Wheat grains of Nadezhda and Kazakhstanskaya 126 varieties were processed by phosphoric acid ( $H_3PO_4$ ) in 5-10% aqueous solutions. For that different concentrations of phosphoric acid: 0.01; 0.1 and 0.5% were tested. Wheat grains were then incubated in a solution of appropriate concentration.

M1 and M3 mutants obtained in the process of  $CdCl_2$  4 varieties of soft wheat of local selection – Shagala, Kazakhstanskaya 3, Zhenis, Lutescens 32 served the objects of the study. The modified plants subsequently laid in the form of lines (A-1, A-2). During the experiment, we used following methods: cytogenetic, hybridological, statistical and morphological.

Cytological studies were carried out at press time preparations using a microscope LOMO Mikmed-1. Genetic analysis of  $F_1$  hybrids and  $F_2$  conducted qualitative and quantitative traits of wheat. Statistical analysis was limited to the determination of the arithmetic mean and its error of the analyzed quantitative traits and to determine the reliability of the difference between the arithmetic means using the Student's *t* test (*t*), a genetic – finding a significant value  $\chi^2$  [12]. Accounting of chromosomal abnormalities in MI, AI and AII of meiosis was performed on temporary acetocarmine preparations under the microscope MBI-3. The representativeness of research result was provided an adequate sample size - 60-100 plants.

**Results and their discussion.** *Genetic analysis of mutant wheat.* Chemical mutagenesis in plant selection is used as an effective method to enhance the variability of the starting material. In the world literature there is sufficient information about the creation of commercial varieties, which derived from

experimental mutagenesis. To use the selected mutants in selection process is necessary to examine their genetic nature. For this, in genetic research are using two methods: analyzes and reciprocal crosses.

*Analyzing cross.* In order to establish the nature of any mutational change by variables usually used carrying reciprocal crosses between the original form and receiving on the basis of its mutant subsequent analysis of the hybrids  $F_1$ . In our studies in  $M_2$  generation plants modified in a number of quantitative and qualitative characteristics was preserved the properties displayed in  $M_1$ . To establish the homo and heterozygous genotype of mutant plants was carried out analyzing cross with an initial variety. Mutant forms with signs of anthocyanin coloration of the stem, pubescent leaf surface, lengthening with spike crossed with an initial variety of Kazakhstanskaya 3 [13]. In  $BC_1$  splitting signs to change and corresponds to the normal ratio of 1:1, and in  $F_2$  is 3:1 ( $\chi^2 = 1.89$ ). Similar results were obtained with the mutant varieties of Shagala with coloration of the stem and leaf axils by anthocyanin. Hybrids  $BC_1$  and  $F_2$  were observed splitting on the grounds of lengthening the stem and normal nodes in the ratio of 1:1 and 3:1, respectively, which indicates that the heterozygous nature of the mutant and monogenic inheritance of this trait. In contrast, cleavage by productive tillering, length and density of the spike in  $BC_1$  corresponded to 3:1, and a  $F_2$  population of 15:1, 13:9 and 3:7, respectively. This shows that symptoms of mutant lines are inherited by a polymer, and complementary mechanisms of epistatic interactions non-allelic genes. This shows that the reaction of plants for the chemical compounds depends on the genotype of wheat

Table 1 – Genetic analysis of  $F_2$  and  $BC_1$  hybrids by crossing mutants with variety Kaz. 3

Characteristicsofmutants	The ratio of altered (modified) and normal plants					
	$BC_1$			$F_2$		
LINE 1						
The length of the spike	27:25	1:1	0.06	188:57	3:1	0.40
Beardlessspike	32:29	1:1	0.04	168:48	3:1	0.89
Anthocyaninsstem	10:13	1:1	0.20	126:32	3:1	1.89
Pubescencesheet	8:10	1:1	0.20	112:28	3:1	1.87
LINE 3						
Crankedstem	22:20	1:1	0.90	118:31	3:1	1.38
Tilleringofplants	45:13	3:1	0.20	120:5	15:1	1.14
The length of the spike	45:18	3:1	0.42	223:51	13:3	0.003
Anthocyanin color of sheet leaves	19:23	1:1	0.38	97:29	3:1	0.26
The thickness of the spike	33:31	1:1	0.06	85:54	9:7	1.38

Further studies had shown that the arising changes in  $M_1$  by the elements of productivity of the varieties Kazakhstanskaya 3, Shagala appeared in subsequent generations  $M_2 - M_6$ . It was proved to conduct reciprocal crossing, where the modified attributes are inherited independently from direction of the crossing. Phenotypic variation of plants was accompanied by a violation of the process of meiosis.

*Cytological analysis of mutant plants  $M_2$ .* Chemical mutagens because of its ability to induce a higher frequency of mutations are used in many countries around the world to create a breeding material. Chromosomal aberrations and violation of cell division during meiosis is one of the major test for mutagenicity of various influences. The most notable in this regard is a meiotic cell division, especially in subjects such as wheat, having a large number of hard identifiable chromosomes. Moreover, violations, reaching the meiotic division, are more likely to be transmitted to the next generation. Mutant plants generation  $M_2$  percentage of damaged cells into  $M_1$  meiosis was 35, and at anaphase AI and AII - 20, which indicates a significant reduction in percent disorders cells compared with mutant plants  $M_1$  (64% AI and 68% - A II) [14]. Violation of phenomenon is cytomixis – the transition of contents to neighboring cells,  $M_1$  amounted 20-30% of all the studied cells, while  $M_2$  the percentage of such cells decreased to 7-9%. So, the percentage of abnormalities in mutant forms of Kazakhstanskaya 3 variety in  $M_2$  was 55%, in contrast, violation that noted in generation  $M_1$  - 90-95%.

The same decrease in the percentage of violations observed by mutants of varieties Zhenis, Lutescens 32 and Shagala. In AI and AII, as well as observed in exercise book some minor violations as a lagging chromosome fragments on the pole, bridge, asynchronous division. Bare cells were occasionally observed [14].

*Cytological analysis of mutant plants M3.* To characterize meiosis in mutant lines M3 and identification monosomic, disomic plant in F1 hybrids with the mutant P1, seen 1080 cells. The results of cytological analysis of mutant plants M3 are shown on Figure 1. As seen in Figure 2 the proportion of cells with pyknosis mutants M3 line L1of Kazakhstanskaya 3 variety was 0.29; mutant of variety Zhenis - 0.10; Lutescens 32 - 0.23; line - L3 variety Shagala - 0.21 compared with impaired cell M1 (respectively). The proportion of cells with univalents was respectively: 0.19; 0.009; 0.16. So, in the older generation of mutants (M3) the variety Kazakhstanskaya 3 and Shagala, selected for practical selection, the proportion of cells with impaired in M1 meiosis is much reduced with mutants like M1 and M2. Violations in meiosis M2 plant from the above varieties have the same character as the M1 plants in meiosis. Typical violations of mutant progeny plants M1-M3 were pyknosis; offset spindle metaphase I; availability univalents, polyvalent of micronuclei in exercise books; asynchronous cell division in AI [14-15].

Comparative study of the effect of different concentrations of phosphoric acid has shown that 5% is set as the optimum concentration of the substance to study the ontogeny and cell division activity of root meristem of wheat germ. Effect of chemical compounds has been considered previously in studies of different directions. However, the genetic basis of variations in plant re-action to the action of these compounds has not been studied. Below is the data for the study of reactions of treated grains under laboratory conditions (Table 2).

Table 2 – Study of cell division and aberrations in anaphase of mitosis

Mutagenandits concentration, %	Total number of analyzed cells	Aberrations	The average percentage of affected cells
Kazakhstanskaya 126			
Control	750	5	0.66±0.01
H <sub>3</sub> PO <sub>4</sub> 0.1%	750	8	1.00±0.01
H <sub>3</sub> PO <sub>4</sub> 0.01%	750	11	1.40±0.01
H <sub>3</sub> PO <sub>4</sub> 0.5%	750	29	3.80±0.40
Nadezhda			
Control	750	3	0.40±0.01
H <sub>3</sub> PO <sub>4</sub> 0.1%	750	14	1.86±0.02
H <sub>3</sub> PO <sub>4</sub> 0.01%	750	17	2.26±0.01
H <sub>3</sub> PO <sub>4</sub> 0.5%	750	37	4.9±0.04

The treated grains were sown in test sites. Pheno-logical observations showed that high level of mono phosphorus (5%) leads to a weak mutagenic effect, while its 0.1% concentration contributes to the development of the biomass. Weak mutagenic effect of 5% concentration is apparently linked to a strong acidification of pH. This is proven by some aberrations of chromosomes in mitosis and meiosis disturbances in plants treated with H<sub>3</sub>PO<sub>4</sub>. Mitosis in mutant plants was accompanied by a massive stick-ing of chromosomes (pyknosis) and offset spindle of metaphase plate (Figure 1).

Mutagenic effect and its importance in the breeding are determined by the results of the mitotic activity and the nature of the aberrations in cell division. They allow us to determine the degree of variability in plants obtained by the action of chemical and physical factors.

The inducing activity of a specific phosphoric acid concentration (5%) on grain germination, cell division and aberrations in mitosis of meristemetic cells of test options compared with control obtained by the action of H<sub>3</sub>PO<sub>4</sub> was studied. The action of the different concentrations of the chemical compound (H<sub>3</sub>PO<sub>4</sub>) was observed within the plant ontogeny. Thus, 0.1% H<sub>3</sub>PO<sub>4</sub> concentration has a minor deviation (1.00 ± 0.01) on the normal course of mitosis compared to control (0.66 ± 0.01) [17].



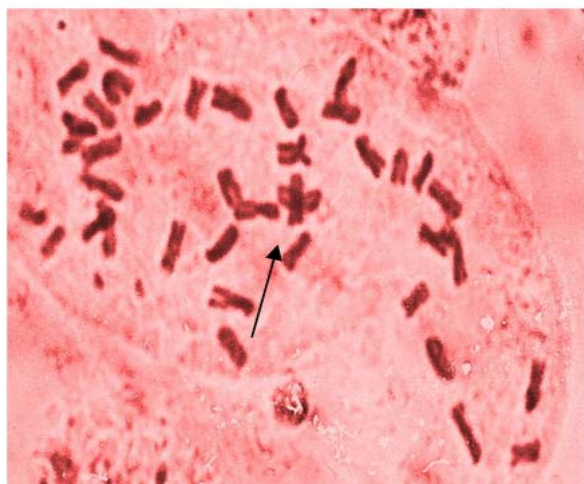


Figure 1 – Mitosis of mutant plants, ditelocentrics are indicated by arrow (x40)

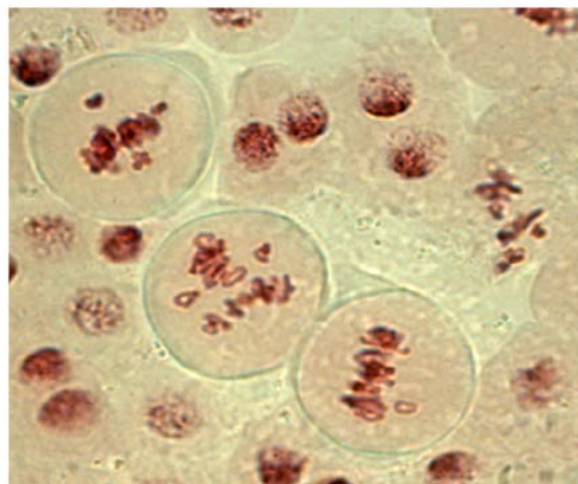


Figure 2 – Sticking of chromosomes in plants, treated with phosphoric acid (x40)

Structural analysis of elements of productivity isogenic lines revealed three lines – IL-Hg, IL-BgHg and IL-Pc, differing significantly by increase of productivity indicators of the spike and 1000 grain weight in comparison with control. Isogenic line IL-Hg with hairy spike can be morphologically well tested during the heading stage and has more saturated color of glume compared to the control. Indicators of spike productivity and weight of 1000 grains of the line IL-Hg was significantly higher than such in control (Table 3).

The length of the spike averaged  $13.0 \pm 0.2$  cm. with the number of spikelets counting  $20.0 \pm 0.4$ . The number of grains in the main spike counts  $63.2 \pm 1.0$  with a weight of  $2.9 \pm 0.1$  g. Grain is medium size, oval with shallow groove. The average value of the weight of 1000 grains was  $48.1 \pm 1.4$  g, in comparison with control –  $44.7 \pm 0.7$  g.

Isogenic line IL-BgHg has a hairy, black ear. The median length of the spike in IL-BgHg line was  $13.1 \pm 0.1$  cm. The number of spikelets on average counts  $20.0 \pm 0.1$ . the number of grains  $65.4 \pm 0.2$ , which was significantly higher than control. Grain size is medium, the groove is not deep. The weight of 1000 grains in line IL-BgHg significantly exceeds such in control counting  $49.7 \pm 0.3$  g ( $P < 0.001$ ). Observed increase in spike productivity indices in lines IL-BgHg and IL-Hg can possibly be associated with the presence of a dominant allele of glume pubescence Hg in these lines. Isogenic line IL-Pc is characterized by the purple color of straw. The length of the spike in line IL-Pc in average is  $12.5 \pm 0.5$  cm. Number of spikelets  $19.6 \pm 0.2$ , number of grains  $63.2 \pm 1.0$ . Weight of grain from the main spike in average is  $2.7 \pm 0.1$ . Major grain has articulate groove. The weight of 1000 grains in line IL-Pc is  $48.4 \pm 0.6$  g, deviation from control is significant under at  $P < 0.001$ . The increase in the average weight of grain from isogenic line IL-Pc is confirmed by the improved grain filling. This can possibly be associated with the increase in productivity of photosynthesis, due to the intensification of this process in anthocyanin containing plants.

Table 3 – Elements of productivity of spike of morphologically marked isogenic lines

Variety/line	Productivity of the main spike				
	Length of spike, cm	Number of spikelets, pc.	Number of grains, pc.	Weight of grain, g	Weight of 1000 grains, g
Kaz. 126	$12.2 \pm 0.1$	$19.0 \pm 0.3$	$51.7 \pm 1.6$	$2.4 \pm 0.1$	$44.7 \pm 0.7$
IL-Hg	$13.0 \pm 0.2^{***}$	$20.0 \pm 0.4^{**}$	$63.2 \pm 1.0^{***}$	$2.9 \pm 0.1^{***}$	$48.1 \pm 1.4^{**}$
IL-Pc	$12.5 \pm 0.5$	$19.6 \pm 0.2$	$63.2 \pm 1.0^{***}$	$2.7 \pm 0.1^*$	$48.4 \pm 0.6^{***}$
IL-BgHg	$13.1 \pm 0.1^{***}$	$20.0 \pm 0.1^{***}$	$65.4 \pm 0.2^{***}$	$3.0 \pm 0.1^{***}$	$49.7 \pm 0.3^{***}$

Deviation from control is significant under \*  $P < 0.05$  \*\*  $P < 0.01$  \*\*\*  $P < 0.001$ .

Earlier upon action with 0.01% aqueous solution of cadmium chloride as a mutagen on the variety Kazakhstanskaya 3 and Shagala mutant lines: L1, L2 and L3 were selected. Mutant forms have long spikes, elongated glumes, glassy large grain, anthocyanin coloration of the stem and leaf axils eyelets, as well as the high weight of 1000 grains. Several lines were higher and thicker culm, thickening and lengthening of the stem nodes, increased productive tillering. These selection and mutant forms of the important features, firmly inherited from generation to generation (M1-M6). In this regard, one of the objectives of this study is to localize genes responsible for economic-valuable signs of mutant forms. It is known that the elongation glumes spike was positively correlated with elongated grains. This fact is a direct proof of the high productivity of the ear mutant form A1. Under natural conditions, when intraspecific hybridization to obtain such form is rarely possible. Therefore, before using a mutant in order to hybridization it was necessary to genetically examine this property of wheat using the method of chromosome engineering. To carry out this work obtained F<sub>1</sub> hybrids seeds using a variety of monosomic lines Kazakhstanskaya 126 in the amount of 22 crossing combinations. Comparative monosomic analysis of the hybrids on the basis of extension glume will be held study of F<sub>1</sub> offspring based on harvest of 2013. Identification of mono- and disomics of Kazakhstanskaya 126 variety served as pre-requisite for this work [17].

*Interspecific hybridization.* Tetraploid endemicspecies *Triticum timopheevii* Zhuk. (genetic formula AtAtGG) is characterized by a unique gene pool controlling resistance to many diseases of wheat. Creating and intensive involvement in the selection process of wheat donors with effective Lr-resistance genes transmitted from wild relatives could significantly expand its genetic basis for one or the other economically valuable traits. High performance pollinating (up to 90 spikes per hour) by native pollen permitted analysis of the actual compatibility of the initial parental forms. Table 4 shows the results of cross-species hybridization.

Table 4 – Fertility of reciprocal hybrids of distant hybridization

#	Combination of breeding	Number of		Percentage of grain folding, %
		pollinated flowers	folded grains	
<i>Soft wheat x T. timopheevii</i>				
1	F <sub>0</sub> ( <i>T. timopheevii</i> x Nadezhda)	190	119	62.63
2	F <sub>0</sub> (Nadezhda x <i>T. timopheevii</i> )	72	11	15.28
3	F <sub>0</sub> ( <i>T. timopheevii</i> x κ-2780)	150	61	40.67
4	F <sub>0</sub> (κ-2780 x <i>T. timopheevii</i> )	56	6	10
5	F <sub>0</sub> (32 shortst. x <i>T. timopheevii</i> )	56	0	0
<i>Soft wheat x T. dicoccum</i>				
1	F <sub>0</sub> ( <i>T. dicoccum</i> x Nadezhda)	282	181	64.18
2	F <sub>0</sub> (Nadezhda x <i>T. dicoccum</i> )	156	41	26.28
3	F <sub>0</sub> ( <i>T. dicoccum</i> x κ-2780)	150	71	47.33
4	F <sub>0</sub> (κ-2780 x <i>T. dicoccum</i> )	130	14	10.77
5	F <sub>0</sub> ( <i>T. dicoccum</i> x 32 shortst.)	32	17	53.12
6	F <sub>0</sub> (32 shortst. x <i>T. kiharae</i> )	33	0	0
<i>Soft wheat x T. kiharae</i>				
1	F <sub>0</sub> ( <i>T. kiharae</i> x Immune1498)	84	34	40.47
2	F <sub>0</sub> (Immune1498 x <i>T. kiharae</i> )	108	12	11.11
3	F <sub>0</sub> ( <i>T. kiharae</i> x κ-2780)	32	17	53.12
4	F <sub>0</sub> (κ-2780 x <i>T. kiharae</i> )	102	17	16.66
5	F <sub>0</sub> ( <i>T. kiharae</i> x 15/20977)	18	8	44.44
6	F <sub>0</sub> (15/20977 x <i>T. kiharae</i> )	118	14	11.86
7	F <sub>0</sub> ( <i>T. kiharae</i> x Nadezhda)	50	29	58
8	F <sub>0</sub> (Nadezhda x <i>T. kiharae</i> )	52	10	19.23
9	F <sub>0</sub> (USA18 x <i>T. kiharae</i> )	48	4	8.33
10	F <sub>0</sub> ( <i>T. kiharae</i> x USA18)	22	12	54.54
11	F <sub>0</sub> ( <i>T. kiharae</i> x USA19)	24	12	50
12	F <sub>0</sub> (USA19 x <i>T. kiharae</i> )	82	23	28.05

*Hybrids with T. timopheevii.* Experimental data shown in Table 4 suggests that the hybridization with different wild cultures of wheat species is successful. However, tying of grains in various combinations ranges from 0 to 64.18%. Apparently, the percentage depends on the genotype of variety from which samples were taken for cross-breeding, as well as from the crossing direction. Thus, the percentage of successful crosses of *T. timopheevii* with soft wheat is relatively high in the case, when the wild form is taken as the parent form. Depending on the number of successfully pollinated spikes the number of hybrid progeny grains varies. Compatibility level of *T. timopheevii* with soft wheat variety Nadezhda is relatively high, and the average is about 62.63%, k-2780– 40.67%, and the percentage of backcrossing luck in hybrid progeny plummets 15.28% and 10%, respectively. F<sub>1</sub> hybrids (*T. timopheevii* to x-2780) of 150 – 61%, and 56 from the reciprocal mating pollinated flowers ensued only 10% of the grain.

*Hybrids with T. dicoccum.* F<sub>1</sub> hybrid offspring with wild species *T. dicoccum* with soft wheat was similar to the results of previous combinations produced with *T. timopheevii*. It is interesting to note that in this case the percentage of luck is much higher than, those combinations where variety Nadezhda served as a father. Thus, from 282 pollinated flowers luck percentage was 64.18%, and in the reciprocal crosses from 156 pollinated flowers tie a percentage of grains appeared 26.28%. Percentage of luck in direct (*T. dicoccum* x k-2780) crossed with the sample to 2780, amounted to 47.33% and -10.77% in reverse [17].

*Hybrids with T. kiharae.* Hybrids of wheat with *T. kiharae*, less productive than the hybrids with the previous combinations. However, in this case, there is a sharp drop in interest luck compared with those combinations where the parent form is taken *T. kiharae*. For example, the percentage of good luck in the forward mating ranged from 54.54% to 40.47% and 28.05% from reverse to 8.33%. This variety of indicators can be explained by the genotype – by environmental conditions for growing plants.

Thus, the study of reciprocal hybrids F<sub>1</sub>, obtained by crossing wheat with wild species – *T. timopheevi*, *T. dicoccum*, revealed clear differences in the percentage of grain formation. In plants, a hybrid combination with *T. timopheevi*, *T. dicoccum* and *T. kiharae* cytoplasm wheat, under which the mother plants as soft wheat has been used, the percentage of luck somewhat lower compared to the hybrids, which served as the parent form of wild species. Hence, one can adopt clearly that the use of the wild-type form as maternal genomes increases compatibility than in the opposite mating. For hybrids derived from crosses with *T. timopheevi* soft wheat characteristic heteroplasmic condition: simultaneously present copies of the wild (the parent) and wheat (paternal) types [17]. Moreover, interspecific hybrids showed high resistance to fungal diseases. However, the instability of the genome of interspecific hybrids requires backcrossing, continuous monitoring of the number of chromosomes in the hybrid offspring and identifying of stable introgressive lines with 42 chromosomes.

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#### **ЖАСАНДЫ МУТАГЕНЕЗДІ ҚАЗАҚСТАНДЫҚ БИДАЙ СЕЛЕКЦИЯСЫНДА КОЛДАНУ**

**Аннотация.** Физикалық факторлардың мутагендік әсері және химиялық заттың шығу тегі (мысалы, азиридин, этиленмин, нитрозометилмочевина, нитрозоэтиленмочевина) селекциялық мақсатта тұқым қуалаушылық өзгерістіктің өсуіне алып келеді және олардың толығырақ зерттелуін талап етеді. Антропогендік факторлардың экологиялық әсерінің зерттелуі, белгілі бір химиялық элементтер арасындағы байланыстары мен комбинациялар бұзылысына алып келе алатын, топырақтағы ауыр металдар концентрациясының жоғарлауына, ауыр металдың мутагендік және токсиндік құрамын танып білуге ықпал етеді.

Генотипті жақсарту арқылы бидай өнімділігін арттыру ауыл шаруашылығында және экономикада маңызды мәселелердің бірі болып табылады. Қазіргі кезде селекциялық және генетикалық зерттеулердің дәстүрлі әдістерін қолдану, мысалы, қанықтырушы шағылыстыру жүргізу, алшақ будандастыру және экспериментальді мутагенез, генетикалық түрлендірілген және бидайдың жақсартылған түрін алу тиімділігін арттырады [1-5]. Ауыр металдар 5 г/см<sup>3</sup> жоғары тығыздықтағы металдар ретінде анықталады.

Барлығы табиғатта табылған 90 элемент, олардың қауіп дәрежесіне қарай үш сыныпқа бөлінген, 53-і ауыр металдар, ал олардың кейбіреулері биологиялық маңызға ие. Ауыр металдардың жинақталуы, мысалы, кадмий (Cd) қоршаған ортаны ластаудың негізгі себебі болып табылады.

Токсинді металдар ақуыздарды белсендіре алады, белсенді орталықтарды блоктауға немесе аллостериялық өзгерістерге себеп болады. Сонымен қатар, олардың көпшілігі мутагенді өзгерістерді тудыруға қабілетті, ісіктер мен макроскопиялық өзгерістерге әкеледі. Ауыр металдардың токсинді молекулалық механизмі толығымен анықталмаған. Cd – екінші деңгейдегі элемент, өсімдіктердің өсуіне және дамуына теріс әсер етеді. Қоршаған ортаға ол электр станциялары арқылы бөленеді, жылу жүйелері, металл өңдеу немесе транспорт қозғалыстары арқылы, іс жүзінде биодеградациясыз жоғары кумулятивтік әсерге ие. Өсімдіктерге ол тыныс алу және фотосинтез сияқты процестерге әсер етеді, құрамында жоғары мөлшердегі кадмий бар өсімдікте жапырақтың хлорозы байқалады, олардың шеттері қызыл-қоңыр түсті, және өсу қарқынының төмендеуі мен тамыр жүйесіне зиян келтіреді. Cd-дің теріс әсері барлық органдарда құрғақ және жаңа массаның артуымен бірге жүреді. Бірнеше зерттеулер тотығу стресті кадмий токсинділігіне байланысты немесе индукция арқылы оттегінің еркін радикалдарының пайда болуымен, ферментативті және ферментативті емес антиоксиданттарды азайту арқылы болуы мүмкін екендігін көрсетті [6-9]. Екінші жағынан, сапалық астықты түр алуда индуцирленген мутагенезді пайдалану жоғарғы тиімділікті көрсетті. Бұл мақала осы бағытта біздің топпен алынған нәтижелердің жиынтығы болып табылады.

**Түйін сөздер:** селекция, химиялық мутагенез, бидайдың изогенді алмастырылған линиясы.

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### **ПРИМЕНЕНИЕ ИСКУССТВЕННОГО МУТАГЕНЕЗА В СЕЛЕКЦИИ КАЗАХСТАНСКОЙ ПШЕНИЦЫ**

Мутагенное действие физических факторов и веществ химического происхождения (к числу последних относятся, например, азиридин, этиленмин, нитрозометилмочевина, нитрозоэтиленмочевина) приводит к увеличению спектра наследственной изменчивости для селекционных целей и требует их более детального изучения. Исследование экологического влияния антропогенных факторов, способных приводить к нарушению определенных связей между химическими элементами и их комбинациями, повышению концентрации тяжелых металлов в почве, способствуют пониманию мутагенных и токсических свойств тяжелых металлов.

Повышение урожайности пшеницы путем улучшения ее генотипа является одной из наиболее актуальных проблем сельского хозяйства и экономики. В настоящее время использование традиционных методов селекции и генетических исследований, таких как проведение насыщающих скрещиваний, отдаленная гибридизация и экспериментальный мутагенез, повышает эффективность получения генетически модифицированных и улучшенных форм пшеницы [1-5]. Тяжелые металлы определяются как металлы с плотностью выше 5 г/см<sup>3</sup>.

Из общего числа 90 встречающихся в природе элементов, разделенных на три класса по степени их угрозы, 53 считаются тяжелыми металлами, а некоторые из них имеют биологическое значение. Накопление тяжелых металлов, таких как кадмий (Cd) в настоящее время становится основной причиной загрязнения окружающей среды.

Токсичные металлы могут инактивировать белки, замещая металлические кофакторы, блокировать активные центры или вызывать аллостерические изменения. Кроме того, многие из них обладают способностью индуцировать мутагенные изменения, опухоли и вызывать макроскопические изменения. Молекулярный механизм токсичности тяжелых металлов в полной мере не раскрыт. Cd – второстепенный элемент, отрицательно влияющий на рост и развитие растений. В окружающую среду он выделяется электростанциями, системами отопления, при металлообработке или движении транспорта, обладает высоким кумулятивным эффектом, практически без биodeградации. В растениях он влияет на такие процессы, как открытие устьиц, дыхание и фотосинтез, при повышенном содержании кадмия у растений наблюдается хлороз листьев, красно-бурый цвет их краев и прожилок, задержка роста и повреждения корневой системы. Он также может привести к уменьшению абсорбции нитрата от корня до побега путем ингибирования активности нитратредуктазы в побегах. Отрицательное влияние Cd на рост растений сопровождается увеличением соотношения сухой и свежей массы во всех органах. Несколько исследований показали, что окислительный стресс может быть связан с токсичностью кадмия либо путем индукции образования свободных радикалов кислорода, либо путем уменьшения ферментативных и неферментативных антиоксидантов [6-9]. С другой стороны, использование индуцированного мутагенеза показало высокую эффективность при создании форм с повышенной урожайностью, улучшением хлебопекарных свойств, измененным ростом и сопротивляемостью растений. Данная статья представляет собой попытку обобщения результатов, полученных нашей группой в этом направлении.

**Ключевые слова:** селекция, химический мутагенез, изогенные замещенные линии пшеницы.

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**ISSN 2518-1629 (Online), ISSN 2224-5308 (Print)**

<http://www.biological-medical.kz/index.php/ru/>

Редактор *М. С. Ахметова, Т. М. Апендиев, Д. С. Аленов*  
Верстка на компьютере *Д. Н. Калкабековой*

Подписано в печать 07.02.2018.

Формат 60x881/8. Бумага офсетная. Печать – ризограф.

9,4 п.л. Тираж 300. Заказ 1.