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IMMUNOLOGICAL INDICATORS IN PATIENTS WITH CHRONIC PHOSPHORUS INTOXICATION AFTER IMMUNOTHERAPY

Abstract. Studies were conducted on the basis of the medical unit of the Shymkent city production association "Phosphorus". A total of 95 patients with CHPI were examined. Subpopulation of T-helper, T-killers increased. Humoral immunity has been changed. Immunomodulin improves the immune system. After immunotherapy, multi-directional shifts in the concentration of IgM, IgG and IgA were detected. The number of lymphocytes in patients with CHPI of the initial degree of intoxication increased by 12.2%. The subpopulation of lymphocytes CDZ +, CD4 + decreased. The content of immunoglobulin IgG after immunotherapy increased by 34.5%, 55% and 35% compared with the control. Phagocytic activity of leukocytes increased. The rate of completion of phagocytosis increased. Phagocytic index decreased. Thus, immunomodulin stimulates immunopathy. Stimulates hematopoiesis. Effectiveness is noticed.

Key words: T-lymphocytes. T-helper, phagocytosis, immunomodulin, phosphorus.

Introduction. Many xenobiotic substances in the long-term intake of the body have a depressing effect on both specific resistance and cellular and humoral immunity [1].

Currently, the study of hematopoiesis and immune status is of paramount importance, since many pathological processes in humans can be both a consequence and cause of various disorders of the immune system, especially in secondary immunodeficiency States [2].

The aim of the work was to study the state of lymphocyte subpopulations in the blood of patients with chronic intoxication with phosphorus compounds (HISF) of various degrees.

Materials and methods. Blood sampling was carried out from the ulnar vein to the heparinized solution of the medium. Peripheral blood lymphocytes were isolated in ficoll-verografin density gradients the number of T-helpers, T-suppressors and immunoregulatory subpopulations of t-lymphocytes with DZ+, CD4+, CD8+, CD9+ and CD56+ were determined by mono-clonal antibodies in rosette formation reactions. The content of serum IgA, IgM, IgG were estimated by radial immunodiffusion according to G. MAPP et. all. (1965). Phagocytic activity was determined by the method of K. B. Ospanov [3].

The studies were conducted on the basis of the medical unit of Shymkent city production Association "Phosphorus". The study included 95 patients with CPCI, including 35 patients with early stage, S0 moderate and 30 with severe degree CPCI; as a control, were examined 54 healthy workers-builders of Shymkent. As an immunostimulant used the drug immunomodulin [4, 5], which was administered to patients with CPCI composed of conventional therapy daily for 5-10 days. Depending on the degree of intoxication, treatment with immunomodulin with a dose of 0.01 g was: with an initial degree of -5 days, with a moderate degree - 10 days and with a pronounced degree - 15 days.

Results. The content of CD56+ in patients with HYSF after therapy, including immunomodulin, increased with an initial degree of intoxication by 19.9% ($p < 0.05$), with a moderate degree - by 24.9% ($P < 0.05$) and with a pronounced degree of intoxication by 40.8% compared with the indicators before therapy. The relative and absolute content of subpopulation of CD8 + lymphocytes after immunotherapy

decreased with an initial degree of intoxication by 20.2%, with a moderate degree by 25 and 40% in persons with a pronounced degree of intoxication.

Multidirectional shifts in IgM, IgG and IgA concentrations were detected in the blood of patients with HISF after immunotherapy in the determination of immunoglobulins. Thus, the content of IgM after therapy in patients with initial, moderate and severe degree of intoxication DECREASED by 15% 14.6% and 26%, respectively, compared with the control. The inclusion of immunomodulin in the treatment of patients with HISF had a positive effect on the content of lymphocytes. The number of lymphocytes in patients with initial degree of HISF intoxication increased by 12.2%. The most pronounced increase in the number of lymphocytes was found in the appointment of immunomodulin for 10 days in persons with moderate and severe degrees of intoxication, while the level of lymphocytes increased by 41.6% and 106.5% compared with the control. When determining the lymphocyte subpopulations were observed multidirectional changes, in particular, the increase of relative and absolute content of lymphocyte subpopulations: CLE+, CD4+, CD56+, CD 19+ and at the same time, a reduction in the relative and absolute content of CD19+. The content of SDH+ after immunotherapy in patients with initial, moderate and severe degree of intoxication INCREASED by 19%, 30%, 39.9% compared with the control indicators. Absolute and relative content of CD4+ increased in patients with HISF with initial and moderate degrees of intoxication by an average of 20% and 30.2% compared with the control. The greatest increase in the content of CD4+ was established after immunotherapy in patients with a pronounced degree of intoxication, with an increase of 89% on average. The content of DM 19+ in patients with initial and moderate degrees of intoxication increased by 49.7%, and 50.3% compared with the control indicators, and the greatest increase in the relative and absolute number of subpopulations of DM 19+ was established after immunotherapy in patients with HISF with a pronounced degree of intoxication. The content of IgG immunoglobulin in patients with initial, moderate and severe degree of HISF after immunotherapy increased by 34.5%, 55% and 35% compared to the control. The content of IgA after immunotherapy in patients with primary CPCI stepeni decreased by 33%, in individuals with moderate and severe degrees of intoxication, the decrease was 42.9% and 33.4%, compared to benchmarks. Patients with CPCI immunotherapy significantly reduced to intensified treatment of the index paracetamol activity and the index of completeness of phagocytosis of neutrophils with a significant decrease in phagocytic index. Phagocytic activity of leukocytes increased by 58.1% 57.9% and 49.8% in patients with initial, moderate and severe degrees. The index of completeness of phagocytosis increased by 61.8%, 65.8% and 70.5% in comparison with indicators before treatment, the indicators of completeness of phagocytosis in individuals with primary and moderate degree close to that of the control data. The phagocytic index, on the contrary, decreased in persons with an initial degree of HISF once, with moderate and severe degrees of intoxication - by 5.1% and 29.1% compared with pre-therapy.

Thus, the inclusion in the traditional therapy of immunomodulin to some extent leads to the normalization of the content of lymphocytes and its subpopulations.

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СОЗЫЛМАЛЫ ФОСФОРМЕН УЛАНҒАН НАУҚАСТАРДЫҢ ИММУНОЛОГИЯЛЫҚ БҰЗЫЛЫСТАРЫ ЖӘНЕ ОЛАРДЫ КОРРЕКЦИЯЛАУ

Аннотация. Зерттеулер «Фосфор» Шымкент қалалық өндірістік бірлестігінің медициналық бөлімі базасында жүргізілді. СФУ мен аурған 95 науқас зерттелді. Т-хелпер, Т-киллер саны арта бастады. Гуморал иммунитеті өзгерді. Иммуномодулин иммундық жүйені жақсартады. Иммуноотерапиядан кейін IgM, IgG және IgA концентрациясында көп бағытты ауысулар анықталды. Ішкі масштабты интоксикация дәрежесі бар науқастарда лимфоциттердің саны 12,2%-ға артты. CD3 +, CD4 + лимфоциттердің субпопуляциясы төмендеді. Иммуноотерапиядан кейін иммуноглобулин IgG мазмұны 34,5%-ға, бақылауға қарағанда 55 және 35%-ға артты. Лейкоциттердің фагоцитарлық белсенділігі артты. Фагоцитоздың аяқталу жылдамдығы артты. Фагоцитарлы индекс төмендеді. Осылайша, иммуномодулин иммунопатияны ынталандырады. Гематопоезді ынталандырады. Тиімділігі байқалады.

Түйін сөздер: Т-лимфоциттер. Т-хелпер, фагоцитоз, иммуномодулин, фосфор.

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ИММУНОЛОГИЧЕСКИЕ ПОКАЗАТЕЛИ У БОЛЬНЫХ С ХРОНИЧЕСКОЙ ФОСФОРНОЙ ИНТОКСИКАЦИЕЙ ПОСЛЕ КОРРЕКЦИИ

Аннотация. Исследования проводились на базе медсанчасти Шымкентского городского производственного объединения «Фосфор». Всего обследовано 95 больных с ХФИ. Субпопуляция Т-хелпер, Т-киллеры увеличились. Гуморальный иммунитет были изменены. Иммуномодулин улучшает состояние иммунной системы. После иммунотерапии выявлены разнонаправленные сдвиги концентрации IgM, IgG и IgA. Количество лимфоцитов у больных с ХФИ начальной степени интоксикации увеличивалось на 12,2%. Субпопуляция лимфоцитов CD3+, CD4+ снижалось. Содержание иммуноглобулина IgG после иммунотерапии увеличилась на 34,5%, 55% и 35% по сравнению с контролем. Фагоцитарная активность лейкоцитов увеличивалась. Показатель завершенности фагоцитоза увеличивался. Фагоцитарный индекс снизился. Таким образом иммуномодулин стимулирует иммунопоэз. Стимулирует гемопоэз. Замечен его эффективность.

Ключевые слова: Т-лимфоциты, Т-хелперы, фагоцитоз, иммуномодулин, фосфор.

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**MONITORING THE DISTRIBUTION OF TRANSGENES
FROM THE GENETICALLY MODIFIED RAPESEED LINE**

Abstract. Use of the modern and high performance technologies in the agrarian industry has to become the main priority for development of agro-industrial complex of our republic. One of such technologies is the genetic engineering of plants. Creation and cultivation genetically of the modified cultures creates particular scratches, mainly ecological and agrotechnical. As there is a potential possibility of transfer of transgenes to not the modified grades and wild relatives due to repollination. The pollen transfer strongly depends on the geographic location of plants and stay near wild relatives. In the Kazakhstan ecosystem rapeseed has a large number of relatives that favorably affects a possibility of cross-pollination. In this regard decrease in a biodiversity of wild flora and fauna is possible. Also such agrotechnical scratches as decrease in a biodiversity of cultivars, change of inappropriate signs and properties, emergence of super weeds can take place. During the presented scientific work the Kazakhstan's first practical monitoring of transfer of stranger genes from the genetically modified rapeseed to grades of rapeseed and its relatives in the open environment was carried out.

Keywords: rapeseed, rape, mustard, genetic transformation, transfer of genes, gene expression.

Introduction. Genetic engineering of plants is the most effective way to obtain plants with desired properties, which provides access to the endless gene pool, allowing the transfer of genes, both from one plant to another, and from other organisms to plants.

The flow of genes from rapeseed can occur on a cultivated field or through a field over long distances due to the spread of pollen or seeds. Unintentional spread of seeds sometimes occurs many years after harvesting due to the germination of dormant seeds in the soil. It can also occur at a considerable distance from farmed GM due to losses during transportation [20].

Influence of the genetic and modified rapeseed on grades and wild-growing flora is well studied in world practice. Rapeseed is not a rigorous self-pollinator, and the value of cross-pollination can reach 30%. The entomophily given about the quantitative ratio or wind are absent, however both factors are important and, perhaps, insects have key value. Depending on environmental conditions, a grade, design of crops, a land relief emergence and frequency of a stream of genes of intraspecific crossing can change.

It is necessary to conduct in-depth studies of influence of the genetically modified plants on a condition of components of concrete ecosystems as the problem of monitoring of environmental risks of transgene plants more and more becomes aggravated in connection with development of genetic engineering. It is necessary to consider genetic features of everyone of the modified plant new genetically at a release in a surrounding medium and commercial use in specific conditions of this or that ecosystem.

In the presented work transfer of stranger genes from the transformed grades to routine grades and their relatives on the example of rapeseed (*Brassica napus*) was studied. Various, carried-out in the world tests show that the majority of crossings between plants happens at short distances, it is less than 10 m, in certain cases cross-pollination is possible apart in 3 km from donors [6-9].

Objects of researches. Served as objects of researches - a working collection of seeds of summer rapeseed: the grade (*Brassica napus*) with a reporterny gene 2GUS35S115x3GUS received from transgene plants of 2015, grade rapeseed (*Brassica napus*), mustard Sarepta grades of (*Brassica juncea*), rape

of a grade of (*Brassica campestris*) of the Russian selection are received from the All-Russian Research Institute of oil-bearing crops of V.S. Pustovoyt; grades Gedemin and the Viking were received from RUP "NAN Scientific and Practical Center of Belarus for Agriculture". Also wild rape and shepherd's bag (*Capsella bursa-pastoris*). Designs with a target gene 2GUS35S115x3GUS pSS cloned in a binary agrobacteriemic vector based on a commercial vector of pCambia2300, were received from Institute of Molecular Biology and Biochemistry of Aytkhozhin of MAUN RK.

Methods of researches.

Receiving explant of rapeseed for the subsequent transformation. Seeds of rapeseed were sterilized by serial washing in 70% an aqueous solution of hypochlorite sodium sodium/chlorate of five water, 70% alcohol, distilled water (x3). The sterilized seeds were sowed on the environment $\frac{1}{2}$ MS bezgormonalny and are germinated within 7 days. In 7 days hypocotyls and kotiledona separately were sheared off and incubated for 2 days on the MS environment with hormone 2,4D (1 mg/l).

Agrobacteriemic transformation of hypocotyls. Carried out an inoculation with the diluted suspension of agrobacteria (0,8 lakes e. at OD600), pSS containing a binary vector with a target gene 2GUS35S115x3GUS within 10 minutes and a kokultivation on the MS environment with hormone 2,4D (1 mg/l) 2 days. Then hypocotyls transferred to the MS environment with hormone 2,4D (1 mg/l) and tsifotaksimy (500 mg/l) and 14 days with the subsequent transfer on the MS environment with BAP hormones (3 mg/l) and zeatiny (2 mg/l), tsifotaksimy (500 mg/l) and Kanamycinum (40 mg/l) incubated. The incubation on this environment was carried out prior to the beginning of a morphogenesis and an embryogenesis and formation of plants. At formation of plants transferred to the MS environment with BAP hormone (0,05 mg/l), tsifotaksimy (500 mg/l), Kanamycinum (40 mg/l). For acceleration of body height of plants transferred to the MS environment (1% sucrose) with IMK hormone (5 mg/l), tsifotaksimy (250 mg/l) and Kanamycinum (40 mg/l).

Agrobacteriemic transformation of kotiledon. Carried out an inoculation with the diluted suspension of agrobacteria (0,8 lakes e. at OD600) containing a binary vector of pSS with a target gene 2GUS35S115x3GUS within 10 minutes and a kokultivation on the MS environment with hormone 2,4D (1 mg/l) 2 days. Then transferred and incubated on the MS environment with BAP hormone addition (4,5 mg/l) and a tsifotaksima (500 mg/l) within 6-8 weeks, with replacement of the environment each 7-10 days before morfo-and an embryogenesis. Then transferred to the MS environment with BAP hormone addition (0,05 mg/l), and Kanamycinum (40 mg/l) and incubated a tsifotaksim (500 mg/l) before formation of plants. For acceleration of body height of a plant transferred to the MS environment (1% of sucrose), IMK hormone (5 mg/l), tsifotaksimy (250 mg/l) and Kanamycinum (40 mg/l) [13, 14].

In a research used a reference medium of MS (Murashige and Skoog, 1962) [11].

Check of an insert and expression of an expression of a GUS gene. The expression of GUS of a gene was checked by method the developed Jefferson [9].

The transformed cages were incubated in the histochemical buffer (0.1 M the phosphatic buffer, 0.1 M ferrieyanide, 0.1% the Triton of H-100, 10.0 mm of EDTA, 20% a methanol, 1.0 mM 5-bromo-3-indolyl-glucoronide (X-gluc, Clontech)) at a temperature of 37°C within 24 clocks. After that tests were washed out in 70% ethanol before achievement of the complete transparence. The expression of a gene of GUS is estimated visually on intensity of coloring of fabric at blue color. For check of an expression of a GUS gene fabrics from leaves and stalks of plants were taken [11].

Receiving seeds from the transgene plants which are grown up in controlled conditions. Transgene regenerant of in vitro with a reporter gene of GUS were transferred to a soil in controlled conditions at a temperature of 25°C, the illumination of 4 thousand luxury, humidities of 50%, the light period of 16 clocks in days before receiving seeds.

Crops and care of plants in field conditions. Crops of rapeseed, a rape and mustard field conditions were carried out in Almaty region to the terms corresponding to area conditions on depth of 2 cm with the subsequent rolling.

For synchronization of blossoming and larger probability of repollination seeds were sowed in the following order: the first sowed rapeseed, in two weeks of rape and mustard. The experimental sites with crops were systematically weeded and processed against wreckers the cruciferous.

Crops in field conditions of plants of rapeseed, a rape and mustard together with the received seeds in 2015 having the reporter gene GUS gen. Seeds from eight plants having a GUS gene expression were

sowed in natural field conditions of Almaty region together with seeds cultural (rapeseed, rape and mustard) relatives.

Crops were carried out on 29.04.2015. For blossoming synchronization the first were sowed rapeseed, then in two weeks were sowed a rape and mustard. Rapeseed seeds with a reporter gene were sowed in the center of a circle. Rapeseed seeds from the center were sowed, beginning from distance in 0,5 m. Crops of plants were made evenly on all area of a circle with the given radiuses. Seeds of rapeseed, a rape and mustard as potential recipients, were sowed is focused on parts of the world (The North, the South, the East, the West, the Northeast, the Sevor-West, the Southeast and the Southwest). Rape, mustard and rape were sown by 10 meter rays. 11 replications of Chris rapeseed and mustard Rocket, 10 replications of Golden rape. The scheme of crops is submitted in the figure 1.

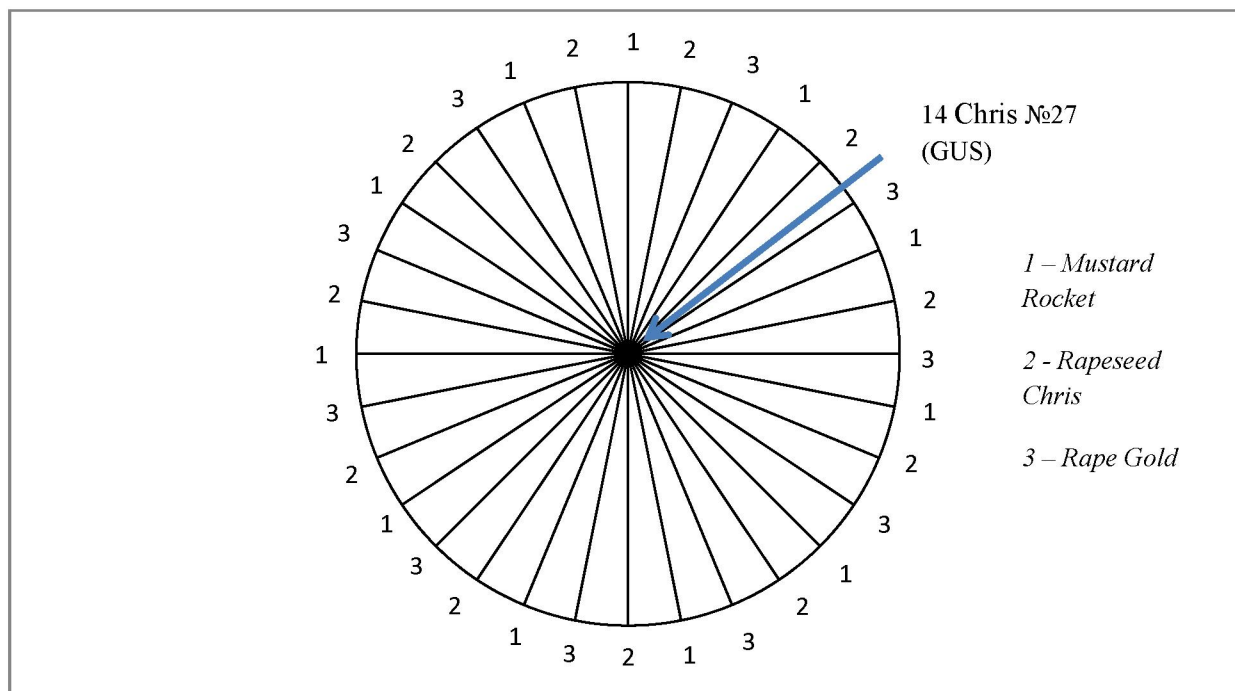


Figure 1 – The scheme of landing of plants on the experimental sites in Almaty region in 2015

Climate in the territory of the experimental site sharply continental. Winters cold and short, summer periods hot and droughty. Long summer. Average temperatures in July are +22 ... +25 degrees. For all calendar year about 250 mm of rainfall drop out. In the area the quite strong winds [gismeteo.ru] blow.

In close proximity with the experimental site there were 10 bee colonies (the North – 1 km), 8 bee colonies (the South – 500 m), 5 bee colonies (the West – 1 km).

Harvesting according to the given scheme and selection of collected seeds on mediums with Kanamycinum. The seed collection was carried out at the following time: rape and mustard - 07.21.2015, rapeseed - 07.29.2015. In 2015 harvesting took place according to the following scheme:

1. Seeds of rapeseed, a rape and mustard were selected through each meter on 5 plants from each beam, carried out marking similarly with rape.

Seeds of each collected plant were threshed and located in paper packages of the above-stated marking, then from each bag took 30 seeds and placed on the selection MS environment with concentration Kanamycinum 70 mg/l and put the corresponding marking and then placed on a prorashchivaniye in the sveto-cultural room on the light mode 16/8 (day/night) at a temperature 25°C and humidities of 50%.

Selection of total DNA from plants [10]. Selection of DNA from plants was carried out with a standard method STAV. Vegetable fabric was placed in a test tube like Eppendorf with a capacity of 1,5 ml; added 700 µl the extraction buffer; added 20 µl RNA elements and mixed, then incubated on a water bath at 60°C within 10 minutes; added 700 µl chloroform and mixed 2-3 seconds. Contents were

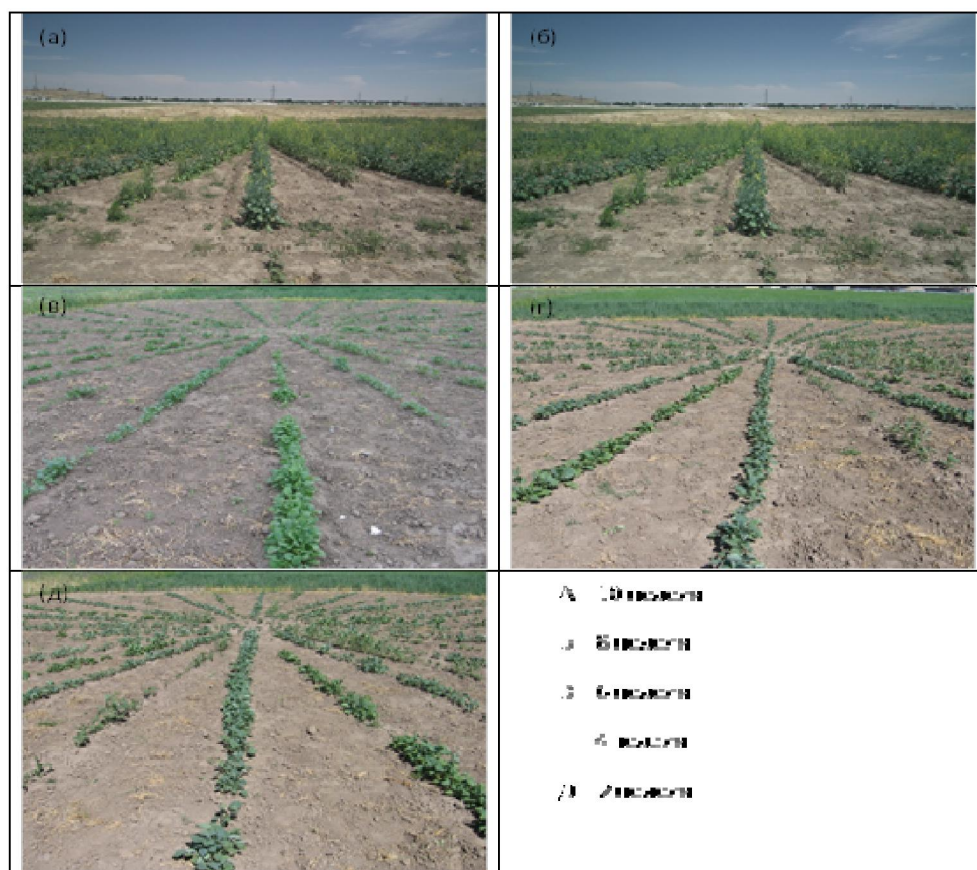


Figure 2 – The state of the field in Uzynagash at different time intervals:
А – 10 week, Б – 8 week, В – 6 week, Г – 4 week, Д – 2 week

centrifuged within 10 minutes at ambient temperature and 14000 rpm. Then a supernatant transferred to other test tube and added 300 μ l isopropanol, mixed, centrifuged within 10 minutes at ambient temperature and 14000 rpm and deleting supernatant liquid.

The deposit was washed out by 70% ethanol twice, as follows: added 800 μ l 70% of ethanol, mixed 1 second, centrifuged 5 minutes at ambient temperature on 14000 rpm. Deleted over sedimentary liquid, dried a deposit at ambient temperature of 10 - 15 minutes.

Added to the dried-up deposit 50 μ l the deionized distilled water, 150 μ l 4M of NaCl, 600 μ l 70% of ethanol, mixed and put in the deep freeze on - 70°C for 20 minutes, then centrifuged 10 minutes at ambient temperature and 14000 rpm, washed out 70% ethanol, deleted supernatant liquid and dried a deposit at ambient temperature of 10-15 minutes, added 30 μ l the deionized water, mixed 1 sec., checked a spectrophotometer and left quality of DNA in the deep freeze on storages at - 200C.

Amplification of DNA by means of the polymerase chain reaction (PCR) [11]. For carrying out the PCR-analysis prepared a reaction mixture of 20 μ l the following structure: 2 μ l the 10th the buffer for a Taq-polymerase, 0,2 μ l 2,5mm the mixes dNTP, on 0,2 μ l mixes of primers, concentration 50 μ mol, 0,2 μ l Taq-polymerases, 16,2 μ l waters.

Exemplars carried out through three corresponding temperature schedules specified in table 1.

Table 1 – Temperature modes of PCR

Stage	Name of a stage	Temperature	Duration
Stage 1	Initial denaturation	94°	5 min., 1 cycle
Stage 2	Denaturation	The Denaturation - 94°C, annealing of primers at 57°C, lengthenings of a chain a polymerase at 72°C	The Denaturation – 30 sec., annealing of primers – 30 sec., lengthenings of a chain a polymerase – 1 min. x 30 cycles
Stage 3	Final polymerization	72°C, then cooling up to 4°C	5 minutes

Products of amplification divided in the elektroforezny camera 1,2% the TAE agarous gel the buffer with addition bromic an etidiya.

Data analysis. Frequency of transfer of a transgene from the transgene site to the corresponding not transgene plants were calculated by division of number of transgene plants on this site on total of the tested seeds on this site and multiplication on total of the seeds which showed a positive take after the analysis of PCR:

$$\text{Frequency transfer of a transgene} = \frac{\text{Quantity of the transformed plants on this site/meter}}{\text{Total of plants on this site/meter}} \times \text{The Common index of percent of transfer of a transgene after the PCR-analysis}$$

Generalization and assessment of results of researches.

Agrobacteriemic transformation of explant of colza a design with a target gene 2GUS35S115x3GUS. According to literary data for *Brassic napus* the revitalization system is adjusted on different fabrics of plants: the microspores [12], hypocotyls [13-15], seven-shares [16, 17], plastids and protoplasts received from sheet disks and kotiledon [18].

Promoter 35S is one of the most widely used, the very strong constitutive promoter responsible for a transcription of all genome of CaMV. It is well-known thanks to its use in transformation of plants. It causes high levels of an expression of genes in plants of bichromatic plants. 35S promoter was called CaMV 35S promoter ("promoter 35S") as the coefficient of a sedimentation of a virus transcript which expression, naturally, is caused by this promoter makes 35S. It was found in the early eighties by Chua and the staff of the University of Rockefeller. The CaMV 35S promoter which is present at many GM-plants is emitted from cauliflower mosaic virus DNA (Cauliflower mosaic virus (CaMV)).

In this experiment by us it was transformed by a target gene of GUS under 35S promoter in a binary vector of pSS - 195 kotiledon and 230 hypocotyls of a grade Chris, 288 kotiledon and 270 hypocotyls of a grade the Viking, 250 kotiledon and 206 hypocotyls of a grade Gedemin. Quantity of the transformed explant and an induction of a morphogenesis and an embryogenesis it is presented in table 2.

Table 2 – Quantity of eksplant and plants

Types of explant	The Grade	Transformed explant	Morphogenesis	Transgene regenerate
Hypocotyls	Chris	230	209	5
	A viking	270	156	7
	Gedemin	206	108	4
Seed leaves	Chris	195	68	3
	A viking	288	144	5
	Gedemin	250	64	2

Apparently from the table 2, the most morfogeny and embriogeny appeared hypocotyls from 50 to 90% of explant, then, as cotyledons from 25 to 50%. At a stage of three sheets regenerant were cloned and a final exit of plants to the transformed explant made 3,4 – 3,7% for hypocotyls and 2,4 – 2,8% for kotiledon.

In the figure 3 the appearance of explant at an embryogenesis stage – after 7 weeks is presented from the moment of an incubation and also transgene plants received on the 12th week after transformation.

Check of an insert and expression of a GUS gene. The received regenerant (table 2) were checked for a gene expression by GUS method the developed Jefferson (1989). As showed the analysis, the gene of GUS was successfully introduced in a genome of hypocotyls of rapeseed of a grade Chris (figure 3). The expression of a gene of GUS was high, judging by visual assessment. Level of an expression could be estimated, observing intensity of coloring.

Receiving seeds from the transgene plants which are grown up in controlled conditions. Thirteen transgene GUS of plants of a grade Chris were transferred to a soil in controlled conditions (figure 5). From 13 plants adapted in a soil 8 which proceeded to be cultivated before receiving seeds.

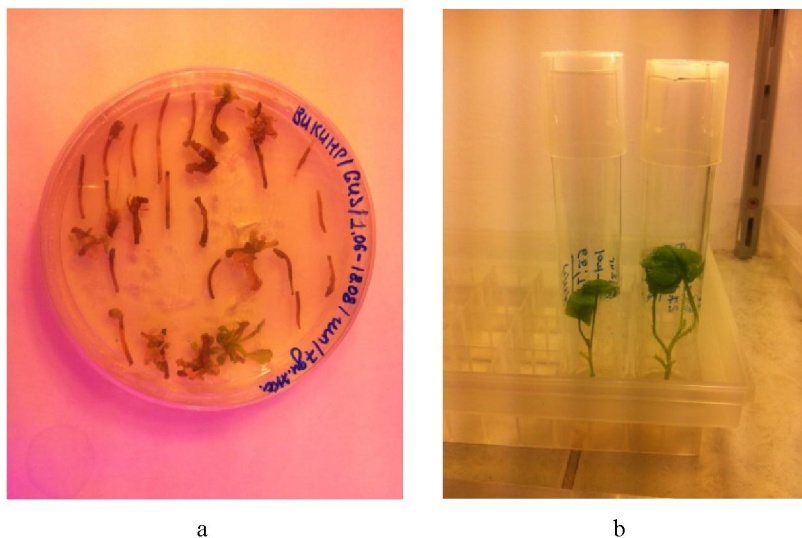


Figure 3 – Appearance of eksplant after 7 weeks from the moment of an inoculation with agrobacteria (A) and regenerant of 12 weeks received later after an inoculation with agrobacteria (B)

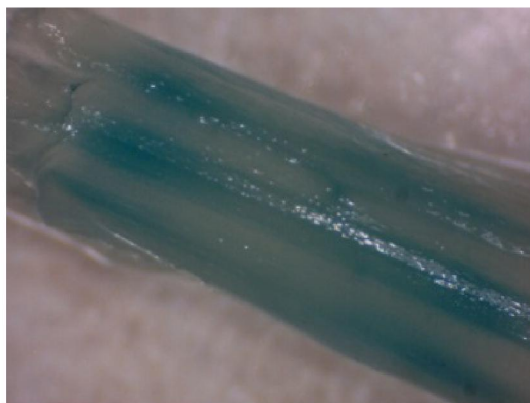


Figure 4 – Expression reporter of a gene of GUS in transgene regenerant of rapeseed of a grade Chris



Figure 5 – Transgene regenerant Chris with a target gene of GUS replaced in a soil in controlled conditions

Seeds of the received transgene plants, were sowed in natural field conditions of Almaty region together with seeds cultural (rapeseed, a rape and mustard) relatives. For blossoming synchronization the first were sowed rapeseed, then in two weeks were sowed a rape and mustard. Rapeseed seeds with a reporterny gene were sowed in the center of a circle. Seeds of rapeseed, a rape and mustard as potential recipients, were sowed is focused on parts of the world.

Harvesting according to the given scheme and selection of collected seeds on mediums with Kanamycinum. In 2015 it was collected exemplars (1 exemplar – seeds from one plant with unique marking): rapeseed – 360, a rape – 322, mustard – 312 (table 3).

Table 3 – Quantity of the exemplars collected from the experimental sites

Kind of plant	2015 год
Rapeseed	360
Rape	322
Mustard	312

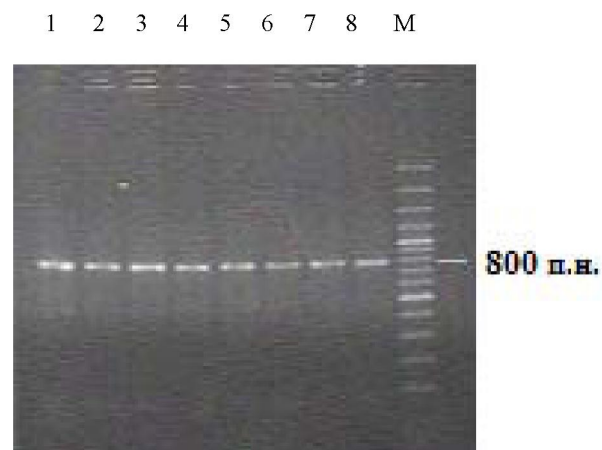
Exemplars of plants which were not tested on the selection environment during the experiment were incinerated.

Definition on presence of an insert and an expression of a transgene of seeds of colza, the surepitsa and mustard gathered during the experiment.

GM of a plant receive by different methods of transformation. Usually for transfer use a plasmid which contains a gene which work gives to a plant the given properties, promoter which regulates inclusion of this gene, terminator of a transcription which contains the selection gene of resistance to an antibiotic to Kanamycinum. Kanamycinum is often used as the selection marker for transgene plants.

For identification of the transformed cages it is necessary to be able to find the stranger DNA integrating into genomic DNA of a plant. All this the reporters of genes which allow or to make selection of the transformed cages demands application, or to estimate activity of the enzyme coded by them. In this work the main stages of screening of this culture on the selection environment are considered and optimized. The way of screening on the selection environment with Kanamycinum is offered. It is picked up optimum concentration for seeds of rapeseed of 70 mg/l, and for seeds of mustard and a rape of 50 mg/l. Thus, the molecular and biological analysis showed that not all survivors when processing by plant Kanamycinum with an insert of genes of GUS in a genome.

Seeds of the plants which passed a test on the selection environment were couched, was emitted DNA for the subsequent definition of an insert. The design under 35S promoter therefore for definition of an insert in estimated plants recipients the following primers for definition of presence of the sequence of this promoter were taken was used for rapeseed transformation: direct - 5-ACTCTGAAAACGGGTCGATA-3', inverse 5-CATCAATCCACTTGCTTTGA-3', at these sequences of primers length of PCR of a product equaled 800 couples of nucleotides.



1 exemplars of rapeseed taken from distance of 10 meters from the center of a circle, 2 - the rapeseed exemplars taken from distance of 9 meters from the center of a circle, 3 - the rapeseed exemplars taken from distance of 6 meters from the center of a circle, 4 - the rapeseed exemplars taken from distance of 3 meters from the center of a circle, 5,6 - the rapeseed exemplars taken from distance of 1 meters from the center of a circle, 7, 8 – monitoring (GUS gene), M – a marker.

Figure 6 – Results of an electrophoresis in 1,2% - number agarous gel of the DNA products of rapeseed (NE direction) received by the PCR method containing the sequence 35S of promoter (exemplars from the field)

In the figure 5 the fact of transfer of a gene of GUS from the rapeseed transformed to routine grades is shown.

In 2015 screening on Kanamycinum of 312 exemplars of a rape, 322 exemplars of mustard and 360 exemplars of rapeseed from the experimental site in 2015 is carried out. From 312 exemplars of a rape underwent screening on the selection environment with Kanamycinum 122 exemplars. Further PCR of these exemplars with the primers specified earlier therefore 38 plants showed a positive take on presence of the sequence 35S of promoter was carried out. From 322 exemplars of mustard underwent screening on Kanamycinum of 129 exemplars which were checked for PCR similarly with exemplars of a rape, from them 63 exemplars showed a positive take on presence of the sequence 35S of promoter. 147 plants drove out of 360 exemplars of rapeseed after screening on the selection environment, 78 of them showed existence of an insert of GUS after PCR (table 4).

To define a possibility of transfer of transgenes (%) cases of transfer of transgenes as equals distances from the center of a circle with GM-rapeseed were considered. For determination of probability of transfer of transgenes the quantity of the transformed exemplars on this site/meter was divided into total of exemplars on this site/meter.

Table 4 – Definition of the repollinated exemplars 2015 with transgene rapeseed by screening methods on the selection environments and PCR of the analysis

Type of screening on repollination	Rape	Mustard	Rapeseed
Models in total	312	322	360
After screening on the selection environment	122	129	147
Percentage index of transfer of a transgene after screening on the selection environment	39,2	39,9	40,8
After carrying out PCR of the analysis	38	63	78
The common index of percent of transfer of a transgene after the PCR-analysis	12,4	19,7	21,7

Schematical representation of frequency of transfer of a transgene at exemplars from the field of 2015 is shown in the figure 7. It is shown that with increase in distance from the center of a circle the frequency of transfer of a transgene decreases. The trendliniya which shows decrease in frequency of transfer, with increase in distance also demonstrates to it.

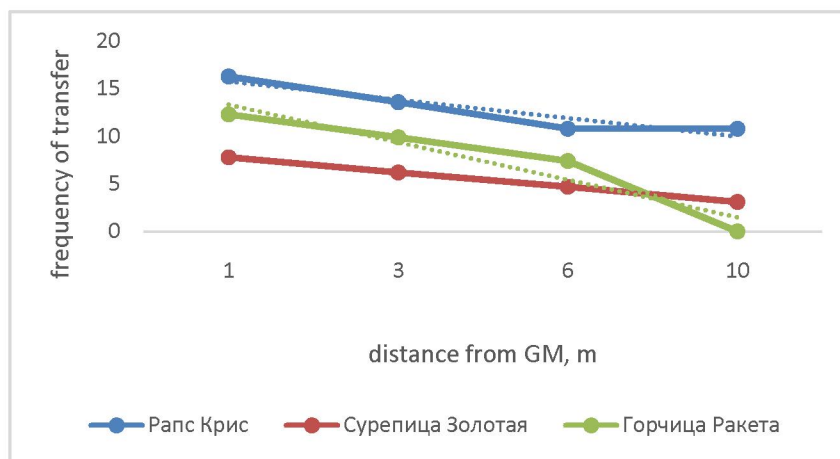


Figure 7 – Frequency of transfer of a transgene at exemplars from the field of 2015 From gold rape GM-rapeseed, mustard the Rocket and to rapeseed Chris

For determination of frequency of transfer of transgenes the possibility of transfer of transgenes on this site/meter was multiplied by the common indicator of transfer of a transgene after PCR – the analysis.

Judging by emergence of events in all parts of the world, without the choice of a dominance of a determinate direction, it is possible to tell that an important role in the course of transfer of a transgene is played by such factors as a wind and insects. It is also possible to judge it from emergence of the isolated and random cases of transfer of transgenes.

In this work in field conditions the opportunity of transfer of transgenes inside between types of the *Brassicaceae* family is shown. There was an exponential decline with increasing distance from the central donor. These results will be coordinated with the results of researches which are carried out worldwide and discussed in the literary review.

For the purpose of improvement of quality of indexes and receiving wider pool of data it is recommended to make an experiment in other areas of the Republic of Kazakhstan and also to increase the scale/area of the experimental site.

Conclusion. In course of execution of the project agrotransformation of hypocotyls and kotiledon of rapeseed by designs with a target gene 2GUS35S115x3GUS cloned in a binary agrobacteriemic vector by pSS based on a commercial vector of pCambia2300 was carried out. Regenerant of rapeseed of three grades were received: Chris, the Viking and Gedemin who were checked for presence of an insert. Check showed presence of an insert at 13 transgene regenerant of a grade Chris from which at transfer in a soil in controlled conditions nine adapted. From seeds of thirteen plants received from regenerant with a GUS gene expression 8 plants which seeds after a prorashchivaniye showed positive test for a GUS gene expression were received.

The received transgene seeds as donors were sowed together with seeds of potential plants of recipients, rape, mustard, rapeseed, a rape of a wild and shepherd's bag on the experimental sites Almaty regions.

The crop according to the given scheme was reaped and screening on the selection environment and PCR the analysis on presence of the sequence 35S of promoter, all exemplars of plants of recipients of a rape and mustard, the Almaty experimental site is carried out.

Results of three-year experiments showed the frequency of possible transfer of genes, high up to 21,7%, for distance to 10 meters from transgene rapeseed to grades of rapeseed (*Brassica napus*) and up to 12,4% of possible crossing with his relatives – mustard (*Brassica juncea*) and a rape (*Brassica rapa*). At the same time, apart to three meters the possibility of crossing with grades and relatives is practically influenced by neither features of year of cultivation, nor a cultivation zone. In too time, perhaps high-quality distinction influencing the free repollination, the bound to blossoming terms and also morphological features of flowers.

Thus results of experiments in open space the Almaty regions showed that transgene rapeseed is freely crossed to not transgene grades and the close relatives of the *Brassicaceae* family (*Brassica juncea*, *Brassica rapa*, *Brassica campestris*). For prevention of cross repollination gene-modified grades need to be sowed separately from routine grades of rapeseed and its close relatives. Besides it is necessary to carry out monitoring of grades of rapeseed and relatives on existence of transgenes at cultivation the inorayonnykh grades with use of the modern methods.

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ГЕНЕТИКАЛЫҚ МОДИФИЦИРЛЕНГЕН РАПС ЛИНИЯЛАРЫНАН ТРАНСГЕНДЕРДІҢ ТАРАЛУ МОНИТОРИНГІ

Аннотация. Біздің республикамызда агроөнеркәсіптік кешенді дамыту үшін аграрлық индустрияда негізгі басымдық болуы керек заманауи және жоғарлы әсерлі технологияларды қолдану қажет. Осы технологиялардың бірі өсімдіктердің генетикалық инженериясы болып табылады. Генетикалық модифицирленген дақылдарды жасап шығару және оларды өсіру белгілі бір қауіпті төндіреді, көбінесе экологиялық және агротехникалық. трансгендердің модифицирленбеген сұрыптарға және олардың жабайы түрлеріне тозандану есебінен таралудың әлеуетті мүмкіндікері бар. Тозанның ауысуы өсімдіктердің географиялық орналасқан жері мен жақын маңдағы жабайы түрлерге өте қатты тәуелді. Қазақстандық экожүйеде рапстың көптеген жабайы түрлері бар және олар алмасып тозандану мүмкіндігіне қолайлы әсерін тигізеді. Осыған байланысты жабайы флора мен фауна алуантүрлілігінің төмендеу мүмкіндігі бар. Сонымен қатар агротехникалық қатерлер орны болу мүмкін, олар еке сұрыптардың алуантүрлілігінің төмендеуі, мақсатсыз белгілердің және қасиеттердің өзгеруі, арамшөптердің пайда болуы. Ұсынылған жұмыс барысында Қазақстанда

бірінші рет ашық ортада генетикалық модифицирленген рапстан рапс сұрыптарына және олардың жабайы түрлеріне бөгде гендердің тасымалдануының практикалық мониторингі жүргізілген.

Түйін сөздер: рапс, сарепт қыша сұрыпы, қышабас сұрыпы, генетикалық трансформация, гендердің тасымалдануы, ген экспрессиясы.

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МОНИТОРИНГ РАСПРОСТРАНЕНИЯ ТРАНСГЕНОВ ОТ ГЕНЕТИЧЕСКИ МОДИФИЦИРОВАННОЙ ЛИНИИ РАПСА

Аннотация. Использование современных и высокоэффективных технологий в аграрной индустрии должно стать главным приоритетом для развития агропромышленного комплекса нашей республики. Одним из таких технологий является генетическая инженерия растений. Создание и выращивание генетически модифицированных культур создает определенные риски, главным образом экологические и агротехнические. Поскольку существует потенциальная возможность переноса трансгенов к не модифицированным сортам и диким сородичам за счет переопыления. Трансфер пыльцы сильно зависит от географического местоположения растений и нахождения вблизи диких сородичей. В казахстанской экосистеме у рапса есть большое количество сородичей, что благоприятно сказывается на возможности перекрестного опыления. В связи с этим возможно снижение в биоразнообразии дикой флоры и фауны. Также могут иметь место такие агротехнические риски, как снижение биоразнообразия культурных сортов, изменение нецелевых признаков и свойств, появление супер сорняков. В ходе представленной научной работы был проведен первый в Казахстане практический мониторинг переноса чужеродных генов от генетически модифицированного рапса к сортам рапса и его сородичам в открытой среде.

Ключевые слова: рапс, горчица сарептская, сурепица, генетическая трансформация, перенос генов, экспрессия гена.

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