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UTILIZING INDUCED MUTAGENESIS IN KAZAKHSTANI WHEAT BREEDING

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Annotation

Mutagenic effect of physical factors and chemical substances (aziridine or ethylene imine, nitrosoethylurea, 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^3. 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. And, this paper is an attempt of summarizing results performed by our group in this direction.

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

ҚАЗАҚСТАНДЫҚ БИДАЙ ШАРУАШЫЛЫҒЫНДА ИНДУЦЦИЯЛЫҚ МУТАГЕНЕЗДІ ПАЙДАЛАНУ

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Аңдатпа

Физикалық факторлардың және химиялық заттардың (азиридин немесе этилен имин, нитрозоэтилмочевина, нитрозоэтиленурея) мутагендік әсері селекциялық мақсатта тұқым қуалайтын өзгергіштік спектрінің ұлғаюына әкеледі, бірақ ол толық көлемде зерттелмеген. Химиялық элементтер мен олардың қосындылары арасындағы белгілі бір байланыстардың бұзылуына, топырақтағы ауыр металдардың концентрациясының жоғарылауына әкелетін антропогендік факторлардың әрекетін экологиялық зерттеу ауыр металдардың мутагендік және токсикалық қасиеттерін зерттеуді жеңілдетеді. Бидайдың генотипін жақсарту арқылы оның өнімділігін арттыру ауыл шаруашылығы мен экономиканың өзекті мәселелерінің бірі болып табылады. Қазіргі уақытта селекциялық-генетикалық зерттеудің дәстүрлі әдістерін, мысалы, бэккросс-селекция, дистанциялық будандастыру, эксперименттік мутагенезді қолдану арқылы бидайдың генетикалық түрлендірілген және жетілдірілген түрлерін алудың тиімділігі артты [1-5]. Ауыр металдарға тығыздығы 5 г/см3 жоғары металдар жатады. Қауіпті дәрежесі бойынша үш класқа бөлінген жалпы 90 табиғи элементтердің 53-і ауыр металдар болып саналады және аз ғана биологиялық маңызы бар. Қоршаған ортада кадмий (Cd) сияқты ауыр металдардың жиналуы қазір қоршаған ортаның ластануының негізгі себебіне айналуда. Улы металдар ақуыздарды инактивациялауы мүмкін, металл кофакторларын ауыстырады, белсенді орталықтарды блоктайды немесе аллостериялық өзгерістерді тудыруы мүмкін. Сонымен қатар, олардың көпшілігі мутагендік өзгерістерді, ісіктерді тудыратын және макроскопиялық өзгерістерді тудыратын қабілеттерге ие. Ауыр металдардың уыттылығының молекулалық механизмі толық зерттелмеген. Cd өсімдіктердің өсуі мен дамуына теріс әсер ететін, электр станциялары, жылу жүйелері, металл өңдеу өнеркәсібі немесе қалалық көліктер арқылы қоршаған ортаға шығарылатын, биодеградациясыз дерлік жоғары кумулятивтік әсерге ие маңызды емес элемент. Өсімдіктерде устьицалардың ашылуы, транспирация және фотосинтез сияқты процестерге әсер етеді, соның салдарынан хлороз, жапырақ орамдары және өсу тоқтауы тамырдың қызаруымен, жапырақтың қызыл-қоңыр түсінің өзгеруімен жүретін өсімдіктердегі Cd уыттылығының негізгі белгілері болып табылады. Ол сондай-ақ өскіндердегі нитратредуктаза белсенділігін тежеу арқылы тамырдан өркенге дейін нитраттың сіңуін азайтады. Өсімдіктердің өсуіне Cd теріс әсері барлық органдарда құрғақ және жаңа масса қатынасының жоғарылауымен қатар жүрді. Бірнеше зерттеулер оттегінің бос радикалының түзілуін индукциялау немесе ферментативті және ферменттік емес антиоксиданттарды азайту арқылы тотығу стрессінің кадмий уыттылығына қатысуы мүмкін деп болжайды [6-9]. Екінші жағынан, индукцияланған мутагенезді қолдану өнімділігі жоғары, сапалы наубайхана, жатуға төзімділік, модификацияланған өсімдік биіктігі мен төзімділігі бар формаларды өндіруде жоғары тиімділікті көрсетті. Және бұл жұмыс біздің топтың осы бағыттағы нәтижелерін қорытындылау әрекеті болып табылады.

Негізгі сөздер: селекция, химиялық мутагенез, изогенді алмастырылған бидай линиялары.

ИСПОЛЬЗОВАНИЕ ИНДУЦИРОВАННОГО МУТАГЕНЕЗА В КАЗАХСТАНСКОЙ СЕЛЕКЦИИ ПШЕНИЦЫ

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Аннотация

Мутагенное действие физических факторов и химических веществ (азиридин или этиленимин, нитрозоэтилмочевина, нитрозоэтиленмочевина) приводит к увеличению спектра наследственной изменчивости в селекционных целях, что, однако, изучено не в полной мере. Экологическое изучение действия антропогенных факторов, приводящих к нарушению определенных связей между химическими элементами и их соединениями, повышению концентрации тяжелых металлов в почве, облегчает изучение мутагенных и токсических свойств тяжелых металлов. Повышение урожайности пшеницы путем улучшения ее генотипа является одной из актуальнейших задач сельского хозяйства и хозяйства. В настоящее время с использованием традиционных методов селекции и генетических исследований, таких как обратный скрещивание, отдаленная гибридизация и экспериментальный мутагенез, повышена эффективность получения генетически модифицированных и улучшенных форм пшеницы [1-5]. Тяжелые металлы определяются как металлы с плотностью выше 5 г/см3. Из 90 встречающихся в природе элементов, разделенных на три класса по степени опасности, 53 считаются тяжелыми металлами и лишь немногие имеют биологическое значение. Накопление тяжелых металлов, таких как кадмий (Cd), в окружающей среде в настоящее время становится основной причиной загрязнения окружающей среды. Токсичные металлы могут инактивировать белки, сдвигая кофакторы металлов, блокируя активные центры или вызывая аллостерические изменения. Кроме того, многие из них обладают способностью вызывать мутагенные изменения, опухоли и вызывать макроскопические изменения. Молекулярный механизм токсичности тяжелых металлов до конца не изучен. Cd – неэссенциальный элемент, негативно влияющий на рост и развитие растений, выбрасываемый в окружающую среду электростанциями, системами отопления, металлообрабатывающими производствами или городским транспортом, обладающий высоким кумулятивным эффектом при практически полном отсутствии биодеградации. У растений он влияет на такие процессы, как открытие устьиц, транспирация и фотосинтез, следовательно, хлороз, скручивание листьев и задержка роста являются основными симптомами кадмийной токсичности у растений, сопровождающимися побурением корней, красно-коричневой окраской листьев. Он также может уменьшить поглощение нитратов от корней к побегам за счет ингибирования активности нитратредуктазы в побегах. Негативное действие Cd на рост растений сопровождалось увеличением отношения сухой массы к свежей во всех органах. В нескольких исследованиях было высказано предположение, что окислительный стресс может быть связан с токсичностью кадмия, либо вызывая образование свободных радикалов кислорода, либо уменьшая ферментативные и неферментативные антиоксиданты [6-9]. С другой стороны, использование индуцированного мутагенеза показало высокую эффективность при получении форм с высокой урожайностью, хлебопекарными качествами, устойчивостью к полеганию, измененной высотой и устойчивостью растений. И эта статья является попыткой суммирования результатов, выполненных нашей группой в этом направлении.

Ключевые слова: селекция, химический мутагенез, изогенно-замещенные линии пшеницы.

Introduction

To expand the boundaries of obtaining primary material in breeding practice, it is promising to use mutant lines that differ from the original variety in valuable characteristics [1].

The purpose of modern plant selection is to adapt the varieties produced for different agroecotypes to the conditions of the external environment as much as possible, that is, to maximize the compatibility between a particular genotype and abiotic and biotic factors of the external environment [2].

For breeding, it is important to produce varieties that are resistant to various stressful conditions. It is profitable for breeders to produce fast-maturing varieties. Because late-maturing varieties face a period of drought from the stage of grain formation, and their quality decreases, and the yield decreases. In addition, spring wheat is late-maturing, and in the northern regions it is subject to autumn cold [3]. Solving these problems is the main goal of breeders. To do this, it is necessary to conduct a genetic study of the nature of rapid maturation of locally adapted varieties. After all, the high quality and grain formation of late-maturing varieties is reduced. It is impossible for all the valuable features of Agriculture to be formed in one variety. There is a reverse correlation between some symptoms. For example, as the yield increases, the quality, compactness and yield of the plant stem, or the increased resistance and quality of the plant to diseases are affected [4-7].

The development and, in particular, rapid maturation of soft wheat depends on certain environmental conditions. To study the role of individual genes that form valuable traits in agriculture, isogenic lineages include the property of rapid maturation, which determines the adaptation of a suitable plant to a particular environment. It is directly related to productivity and is characterized by the ability to get out of adverse external conditions /frostbite, drought, pests, diseases/, as well as fully use favorable conditions. The rapid maturation of soft wheat is mainly facilitated by the Vrnl-3 system [8-10].

Therefore, it is advisable to comprehensively study the type of development of varieties, in local conditions, its interphase length in ontogenesis, combined with the yield of the plant and its resistance to high and low extreme temperatures. Such focused research makes it possible to produce fast-maturing, that is, soft spring wheat with a short maturation period. In this regard, conducting a genetic analysis of the types of fast-maturing varieties, studying the type of development of varieties and lines concentrated in the local selection and gene pool from different foreign countries, is an urgent problem of selection. Therefore, the purpose of our research work is to conduct a genetic analysis of the type of development of mutant lines obtained from zoned and promising breeding varieties.

Research methods and materials

Mutational, hybridological, cytological and monosomal analyses were used as research methods. Local soft wheat varieties as Shagala, Lutescens 32, Kazakhstan 3, Mutant lines from the variety and Monosomal lines of the Kazakhstan 126 variety were used as a material for conducting the experiment, which is widely used in local selection. The materials obtained for the study were treated with cadmium heavy metal salt (CdCl2).

Research results and discussions

The influence of the above-mentioned alkyl chemical compounds on grain crops often leads to morphological variability (Morphosis) and non-formation of chlorophyll grains of the plant (chlorosis), which have no value for selection [11]. Therefore, it is necessary to look for a weak concentration of chemical compounds that do not have a toxic effect on the product, increasing only the limits of plant variability. Despite the interest in the problem of mutations and the huge amount of work done, the genetic nature and mechanism of variability still require sufficient study. Morphobiological and cytogenetic assessment of induced variability in soft wheat varieties under the influence of heavy metal salt [12].

The content of cadmium in the soil and plant was studied by atomic adsorption. During the period of plant development, especially during maturation, the amount of cadmium did not exceed the limit of safe concentrations. However, the occurrence of mobile cadmium in soft wheat varieties of Kazakhstan 3, on light brown soils grafted by Shagala, can be attributed to the fact that they were treated with metal compounds of grain - seeds and with different concentrations of cadmium.

To evaluate the effectiveness of mutagens, the mitotic index and data describing chromosomal disorders are widely used [8; 13-16]. They allow us to assess the degree of variability that occurs in plants under the influence of chemical and physical factors. The growth of the first sprout, induced by a certain concentration of cadmium chloride and zinc chloride (0.01%), cell division activity and structural disorders of chromosomes were studied. The effect of various solutions of chemical compounds on the plant was observed from the first days during the growth of wheat sprouts. The growth of the first sprout of seeds treated with a certain solution of cadmium chloride and zinc chloride - 0.01%, the activity of cell division and structural disorders of chromosomes were manifested by variability in the varieties Kazakhstan 3, Shagala, Zhenis and Lutescens 32.

After treating dry wheat grain with a concentration of 0.1% cadmium chloride, it brought the growth of the Sprout to a lethal state, 0.01% inhibited the growth of the Sprout for 4 days, and in a 0.001% solution, the growth rate of the control grains was the same. On the contrary, a 0.1% vertical solution of zinc chloride inhibited the growth of the Sprout for 1 week, while 0.01% for three days.

Among the studied concentrations, a 0.01% solution of cadmium chloride inhibited the growth of sprouts, causing chromosomal aberrations and morphological variability in cell division. Therefore, a concentration of 0.01% of cadmium salt was obtained as an optimal concentration to expand the limits of variability in wheat.

Solutions of 0.1% and 0.01% zinc salt did not cause significant changes in cell division and morphological features, although they inhibited the growth of sprouts. Grains of all varieties were treated once with a 0.01% solution of cadmium chloride.

To study the activity of meristem cell division in the undergrowth of grain treated with 0.01% solutions of cadmium chloride and zinc chloride of the Kazakhstan 3 Variety, cytological analysis of more than 500 cells of the control variety and each variant was carried out, and the mitotic index was calculated. Among the variants treated with chemical compounds, the average cell division activity in a 0.01% solution of zinc chloride (4.75 ± 0.05) was higher than in a solution treated with cadmium chloride (2.25 ± 0.02) (table a-1). Cell division activity was characterized by low indicators in both variants compared to control (6.61 ± 0.02) (Table 1).

Table 1 Mitotic activity of the cell in meristem tissue of the Kazakhstan 3 variety, depending on the concentration of cadmium chloride and zinc chloride

Variety	Experience	Number of cells viewed	Pronounced mitoses	Percentage of mitotic index	Mitosis stage					
					prophases		metaphases		anaphase	
					number	%	number	%	number	%
Kazakhstan 3	523	177	6,61 ± 0,02	102	0,19	46	0,08	29	0,05	523
2. $ZnCl_2$ 0,01	654	127	4,75± 0,05	63	0,09	26	0,03	38	0,05	654
3. $CdCl_2$ 0,01	549	86	2,25 ± 0,02	15	0,02	31	0,05	40	0,07	549
4. $ZnCl_2$ + $CdCl_2$ 0,1	589	70	0,54± 0,03	35	0,05	24	0,04	11	0,01	589
5. $CdCl_2$ 0,1+ $ZnCl_2$	--	-	-	-	-	-		-	-	-

It has been observed that these chemical compounds have different effects on cell division, despite the fact that they belong to the same series of salts. Under the influence of chlorine zinc, the activity of cell division accelerates, and under the influence of chlorine cadmium, the rate of cell division slows down, that is, it has a harmful effect on the plant. At the same time, the effect of two combined salts on the plant was observed in different directions: in the first direction - after the seeds were first treated with a solution of cadmium chloride in water for 5 hours, washed with distilled water and held for an additional 5 hours in a solution of zinc chloride. In the second direction, on the contrary, chlorine zinc was first obtained for processing. The first of the two directions of treatment left the plant without growth, and the second inhibited the activity of cell division.

The 0.01% concentration of cadmium chloride used in the experiment caused aberrations in meristem cell division: two-to three-nucleated and non-nucleated cells, destruction of achromatin filaments in the chromosome, bridges in anaphase, rings.

Under the influence of cadmium chloride, the growing season in the generation of Kazakhstan 3, M2 Shagala varieties is 15 - 17 days later than the control one.

In the offspring M3 and M4 of the Shagala and Kazakhstan 3 varieties, the duration of the growing season was delayed by 2-3 days compared to the control variant. Although the inhibition of growth and maturation of a plant in ontogenesis is constantly transmitted from generation to generation, it seems that the limits of its manifestation of these properties are subordinated to the state of the external environment.

Only the growing and maturation periods of the Lutescens 32 variety were the same as those of the first variety. The period of full maturity of mutant lines began later with the control varieties.

As a result of phenological observations of the timing of growth and maturation of mutant lineages in M2-M4, it was observed that the rate of development of plants matures late in comparison with the first varieties. Early-maturing mutants included short-stemmed squerheads, compactoids, mustachioed, rare earwigs without whiskers, and late-maturing (4-8 days) cylindrical and dense earwigs. Thus, under the influence of cadmium salt, the germination rate of soft wheat grains was slowed down for 1-2 weeks in the offspring of M1 - M2 compared to the control variants, and in the offspring of M3-M4-for 3-4 days.

The germination and growth of the grain of experimental variants is subordinated to the specifics of the genotype and the state of the environment. During the experiment, it was proved that the effect of heavy metal salt affects the growth and development of the plant at all stages, not just one stage.

For the selection model, it is possible to achieve a high result only by a complex study of various selection parameters of the genetic nature of quantitative and qualitative characteristics in order to produce fast-maturing, disease-resistant, productive, high-quality varieties, and the first valuable material can be sorted for hybridization. Such valuable varieties and isogenous lines in different gene systems allow us to accumulate the gene pool of our country's soft wheat [10-12].

Analytical hybridization of plants with the first varieties with altered features from the varieties Kazakhstan 3 and Shagala was carried out. The difference of the studied features of the Kazakhstan 3 Variety in modified and simple plants is 1:1, and the difference in the ratio of 3:1 in the F2 generation proves the monogenic inheritance of mutant features.

As a result of the analytical hybridization of plants of the Shagala variety with a dense ear and a brownish - ochre color of the leaf blades with the first Variety, the output of normal and altered plants was 1:1 and divided into a ratio of 3:1 in F2, i.e. the mutant trait is monogenic, dominant heredity. In contrast, the result of analytic hybridization from stem binding and ear elongation showed a ratio of 3:1, and divergence in the F2 population showed a ratio of 15:1 and 13:3 (Table 2).

It follows that the presented features of mutant lines are inherited under the influence of complex, non-allelic genes (polygenic and epistasis).

As a result of hybridization of a long (16 cm), dense ear (0.80) plant with a prismatic plant of the control variety (0.50), which changed from the Shagala variety, it was found that its summer type (from the vrn gene) changed to the autumn type (from the Vrn gene). In the summer field, the number of plants that remained in the state of tillage was four, and one of them grew and produced a crop.

Table 2 Differentiation of Altered Traits from Kazakhstan 3 and Shagala varieties in generations VS1, F2

Signs of mutant forms	The relationship between mutants and common plants					
	VS1			F2		
	Factual	Theoretical	$\chi 2$	Factual	Theoretical	$\chi 2$
Line L1						
Ear length	27:25	1:1	0,06	188:57	3:1	0,40
Ear without a mustache	32:29	1:1	0,04	168:48	3:1	0,89
Brown chestnut stem	10:13	1:1	0,20	126:32	3:1	1,89

Leaf hairiness	8:10	1:1	0,20	112:28	3:1	1,87
Line L3						
Branching stems	22:20	1:1	0,90	118:31	3:1	1,38
Number of binding Sessions	45:13	3:1	0,20	120:5	15:1	1,14
Ear length	45:18	3:1	0,42	223:51	13:3	0,00
Leaf petiole dark brown color	19:23	1:1	0,38	97:29	3:1	0,26
Dense earwax	33:31	1:1	0,06	85: 54	3:1	1,38

The offspring of a self - pollinated modified plant proves that the gene responsible for the rate of development of the Shagala variety is heteroallic-consists of the genotype Vrn1Vrn1vrn2vrn2, which is different from the genotype of the Shagala variety, adapted to both autumn and summer conditions.

Thus, under the influence of cadmium salt, it was determined whether the genotype of mutant plants of the Kazakhstan 3 Variety and the dominant inheritance of the altered trait were known as a result of analytical hybridization, and how many genes are inherited by mutant traits in F2.

In the experimental variant of the Shagala Variety, the difference in offspring F2 corresponds to the ratio of stem binding to 15:1, ear length to 13:3, and ear density to 9:7, and the property of non-allelic genes of mutant traits of complex Shagala and Kazakhstan 3 varieties M1 (thickening of the stem joint, knee Binding of the stem, grain shape, ear color, moustache) does not change regardless of the direction of recipient hybridization of the breed.

Experimentally, it is difficult to accurately limit the types of plant development to the autumn and summer types. Most often, this limit is determined by the length of the growing season of wheat, depending on the conditions of the environment in which the experiment takes place. This limit fluctuates depending on weather conditions. Therefore, it is necessary to use a spring wheat variety from the low-expressiveness Vrn2 gene, which accurately determines the summer and autumn limits in an experiment and determine the initial period of germination. The genotype of the spring wheat variety is determined by any dominant Vrn genes, while the winter varieties are determined by the recessive alleles of all dominant loci /Vrnl Vrnl Vrn2 Vrn2 Vrn3 Vrn3 Vrn4 Vrn4 [14].

The next important system is the ppd locus / photoperiod-the different effect of the photoperiod on the change in the duration of the day/. Vrn genotypes provide diversity in the rate of development under certain conditions. It was also found that there is a part of the genetic diversity that is not related to yarovization and photoperiod reactions. It is a system that determines the difference of a particular genotype in the range of 4 to 10 days according to the loci of Vrn and ppd, that is, the rate of development characteristic of the genotype. Recessive loci Vrnl, Vrn2, and Vrn3 from the Vrn system are the last studied genes. They determine the yarovization reaction, which is characteristic of autumn varieties and differs from the PPD genes (Table 3).

Table 3 Growing season of hybridized varieties in selection from the date of growth to the beginning of maturation

Variety	Sowing date	Date of release of the sprout	Date of wheat earing
Kazakhstan 3	15.04	21.04	12.06
Kazakhstan mutant 3	15.04	21.04	10.06
Line 1	15.04	21.04	14.06
Shagala	15.04	21.04	14.06
Line 2	15.04	21.04	21.06
Line 3	15.04	21.04	21.06

A system consisting of one or more dominant alleles according to the Vrn genes partially or completely inhibits yarovization at lower temperatures. Such genotypes are characterized by summer varieties, which also differ in the photoperiod system. Genotypes consisting of two genetic systems /type of development Vrn - photosynthetic ppd/ exhibit two-sided properties and characterize both autumn and summer types of development. Such a homozygous geiotype is dominated by Locus Vrn2, which is characterized by recessive genes from other loci on both systems - Vrnl Vrn2 Vrn3 ppdl ppd2 ppd3 [12].

According to many authors, varieties consisting of the [5,10] Vrn gene do not require springization, they mature quickly. Kazakhstan varieties 3, Line 1, Line 2 consist of the Vrn gene, that is, this locus determines precocity, but they were colossed on June 21 [Table 3l], and the ear varieties showed precocity. Line 2 consists of the Vrn gene, that is, this locus determines precocity, but they were colossed in 21o [Table 3l], and the ear varieties showed precocity. Most likely, these varieties contain the ppd gene, which inhibits colossus. The Ppd locus shows a photo report. This period depends on the length of the day, the gene that affects the inhibition of growth stages, earing. Does not affect any variety. The effect of the Ppd gene depends on the specific genotype [4]. As a result of the study, it was found that the varieties Kazakhstan 3, Kazakhstan mutant 3 consist of the genes Vrn 1, Vrn 3. It was found that the varieties of Seagull consist of the genes Vrnl, Vrn2. Varieties consisting of Rn1, Rn3 genes show rapid maturation. However, they differed from each other in the earing period.

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