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Sources and Donors for Soft Wheat Selection by Resistance to Yellow Rust

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Yellow rust (*Puccinia striiformis f. Sp. Tritici*) – is one of the most spread and most serious wheat diseases and one of the main factors reducing the yield of wheat. Epiphytotic diseases of wheat rust cover the entire continents leading to catastrophic crop failures. In order to control the stability, it is very important to have available molecular genetic markers linked to these symptoms. Using molecular markers *STS, SCAR*, carriers of resistant to yellow rust *Yr18*/*Lr34* gens and effective *Yr10, Yr15* gens were identified. Varieties Karasai, Moro, Naz, Mereke-70, Mereke-75, Sultan-2, Akdan with genom Yr10, varieties Mereke-70, Almaly, Arap, Karasai, Dinara with genom Yr-18 and only sort Raminal with genom Yr15 were identified among 30 investigated varieties. Varieties Karasai and Mereke-70 are the most valuable donors or resistance to yellow rust. Two resistance gens were identified in them. Varieties Karasai, Raminal, Sultan-2 demonstrated higher performances of some productivity elements in comparison with standard variety Almaly in the structural analysis. In order to increase the resistance to yellow rust, these genotypes were proposed to be used as markers in the program MAS (Marker assisted selection).

Key words: Wheat cultivar, stripe rust, molecular marker, resistance genes.

Wheat is one of the most important food crops in the world. Grain production has been and remains an important strategic resource and a basic sector of the agricultural production of Kazakhstan. In recent years, the country produces annually up to 20-25 million tons and exports up to 5 million tons of grain. However, the average yield of grain crops is less than 13-15 kg/ha, which is due to a biotic and biotic stresses, in particular, diseases that reduce yield up to 10-25% or more in the years of epiphytotic development[1]. One of the main reasons for the high post-harvest losses of grain Kazakhstan is the intensive development of fungal diseases. Yellow rust of wheat rust the most common and dangerous diseases of wheat, which cause serious economic damage, reducing the yield and quality of grain. According to experts of the Food and Agriculture Organization of the United Nations (Food and Agriculture Organization - FAO), at the end of the last century, the loss of wheat in the world from diseases reached 33.5 million tons, which is about 10% of the potential yield of the most important food crops[2]. Pathogen *Puccinia striiformis West* causes excessive transpiration, drying of leaves and weakens the process of assimilation in the spike, reduces the activity of enzymes and reduces the weight and number of grains in the spike. Due to the decrease of gluten in components of low molecular weight in grain, which influences on the quality of flour, rust affects

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adversely the baking performance[3]. In most regions of the wheat, the grain loss due to the yellow rust can vary from 10 to 70%, depending on the susceptibility of varieties, the period of infection, duration and level of disease development[4,5]. The cause of disease severity in a wide range of climatic zones is the high variability of the pathogen and its ability to migrate. Race of stripe rust pathogen, which is virulent to gene Yr27, caused serious crop losses in North Africa, West Asia and East Asia during heavy epiphytoties in 2009 and 2010. In 2013, the yellow rust affected the most susceptible species in some areas of Afghanistan, Azerbaijan, India, Iran, Iraq, Morocco, Pakistan, Turkey and Uzbekistan[6-8]. In recent years, phytosanitary situation is deteriorated in Central and Eastern Asia and North Africa, as well in Kazakhstan, in connection with the spread of yellow rust of wheat[9,10]. Five epiphytoties outbreaks of yellow rust took place in the region over the past 15 years (in 1998, 2000, 2005, 2009 and 2010.). That led to significant losses of wheat crop[10]. The pathogen is found in the territory of Kazakhstan almost every year, except for extremely dry years[11]. Favorable climatic conditions (heavy rainfall during the second half of the growing season, coupled with high temperatures and humidity) and the lack of resistance of cultivated varieties contribute to high disease severity of crops. An important element in the plant protection is breeding and cultivation of the adapted, disease-resistant varieties. The creation and introduction of such varieties will reduce crop losses, increase the profitability of seed production and improve production quality[12]. In order to control the disease resistance with more reliability, it is important to have at the disposal of molecular genetic markers associated with this trait. Using the PCR (polymerase chain reaction) has opened up new possibilities for marking the resistance to plant diseases. On the basis of marker assisted selection (Marker Assisted Selection - MAS) it is possible to identify effective resistance genes and track them in the fissile generations of hybrids[13,14]. Up to date, more than 70 genes of resistance to yellow rust are registered in the wheat genes catalog, including 40 identified ones and 30 Yr-genes with temporary designation[15]. Most of them are the dominant, race-specific ones, many of them are not able to protect the variety from infections. Therefore, a search for new sources of genes for resistance to wheat rust is necessary[16]. Up to date, molecular markers are identified for a number of genes of resistance to yellow rust: Yr5[17,18], Yr9[19], Yr10[20,21], Yr15[22], Yr28[23] and other Yr-genes. According to some researchers, the genes of resistance to yellow rust Yr5, Yr10 and Yr15 are effective for Central Asia and Kazakhstan[24, 9]. In this regard, in the present study, attention was drawn to the part of the effective genes of resistance to yellow rust - Yr5, Yr10, Yr15, which were identified in the course of molecular screening of wheat germplasm. One of the main objectives of the field crop cultivation is increase the crops of the harvest. In order to achieve these objectives, the main goal of our work is introduce new wheat varieties, which are resistant to yellow rust, high productive and meet the reqirements of the modern field crop cultivation.

MATERIALS AND METHODS

Isogenic lines of Avoset variety were used in our research for genetic analysis of population of yellow rust agents. Isogenic lines are considered as very convenient objects in the genetic researches, because bases of isogenic lines are identical. The difference of these objects is only one gen, all the other genes are the same. Therefore, it is possible to achieve accurate and reliable results by means of isogenic lines in determination of resistance to yellow rust. 30 varieties of the allowed to industrial cultivation (commercial) and perspective varieties of soft wheat (Triticum aestivum) are used in Kazakhstan. A field study was performed at the experimental station of Kazakh Research Institute of Farming (KazRIF), Almalybak, Almaty region in 2014-2015 cropping season. Molecular-genetic analysis was conducted in the Laboratory of genetics and selection in the Institute of Plant Biology and Biotechnology. Method of McIntosh et. al.[25] which allows to estimate reaction type and affect percentage of the variety was used during the research. According to this method there are 5 types of reaction: 0 - immune, without simptoms of damage. No affects based on physiologic incompatibility of the plant and the pathogen. R-resistant, capable to withstand to pathogen (small, poorly developed seldom uredinidia are surrounded by necrosis); MR- mildly resistant, with

small uredinidia surrounded by chlorosis; MS-mildly susceptible, with medium sized urediniia which covers up to 20-40% of leaves surface; S-susceptible, uredinia are of big size, no chlorosis features, affected area is more than 50%. The international standard - wheat variety Morocco - was used as susceptible control in the field experiments. Extraction of genomic DNA from plant material was performed from 5-day-old wheat seedlings by means of CTAB method[26]. PCR was used for the identification of carriers of resistance genes .The primers used in this study are listed in table 1. The PCR reactions were performed according to the optimized conditions for Yr10, Yr15 and Yr18. The volume of the reaction mixture for PCR was 10 µL, containing 0.5 µL of 10X buffer with Taq-polymerase, 1.5 µL of dNTP, 0.5µL of each primer, 0.5 µL of Taq-polymerase, and 18 µL of MQ-H2O. For the separation of the amplified DNA fragments, we carried out electrophoresis in 1,5 % agarose gel in TBE buffer (45 mM tris-borate, 1 mM EDTA, pH 8)[27]. The PCR amplification was accomplished in a thermal cycler (Eppendorf, Germany). The PCR profile included initial denaturing for 5 min at 94°C, 45 cycles consisting of 1 min at 94°C (denaturing), 1 min at 45°C (annealing), and 2 min at 72°C, and a final of 7 min extension at 72°C (elongation) with some modifications according to the optimized conditions for each resistance gene. The gels were visualized on BIO-PRINT MEGA for documentation of allele sizes in DNA samples.

RESULTS AND DISCUSSION

Search of carriers of genes of resistance to rust was based on molecular screening of wheat samples using primers specific for each gene. The genes of resistance to yellow rust: *Yr10, Yr15,* and *Yr18/Lr34* were identified in the samples.

Table 2 presents the results of molecular screening and phytopathologic evaluation which are done using molecular markers linked to effective resistance genes *Yr10, Yr15,* and genes *Yr18/Lr34* for 30 varieties, resistant to yellow rust and allowed to industrial planting in Kazakhstan. Resistance of varieties to yellow rust was evaluated three times, i.e. every week after first symptoms of disease. Among 30 researched varieties, Alikhan, Egemen, Akdan, Maira, Manshuk, Tungush, Kazakhstan-10 were 0-immunal, without any symptoms of disease. Varieties Azharly, Sultan-2, Intensivnays, Mereke-75 showed resistance between 5R and 10MR, Kyzylbiday showed reaction type 10MS, Raminal and Zhetisu showed 15MS, Egemen-20, Mereke-70, Matai showed 20 MS, Nureke, Dastan, Dinara, Naz showed 30 MS, Keremet showed 40 MS. Varieties Arap, Karasai, Krasnovodopadskaya, President showed reaction type 40S, Almaly and Taza showed 50S, Karligash showed 60S, so these varieties proved to be susceptible. In order to identify carriers of gens of resistance to rust types, molecular screening of wheat samples using primers specific to each primer.

Dominant Yr10 gene was originally identified in the wheat line P.I.178383 and it was localized in the short arm of chromosome 1B, at a distance of 2,0 cM from Rg1 gene controlling glume red coloring[28], and at a distance of 5,0 cM from gene encoding gliadin Gli -1B[29]. The test line of this gene is a French variety Moro. Yr10 gene showed resistance to most existing isolates of *P.striiformis tritici* in China[30] and Kazakhstan[31]. In order to identify carriers of this gene, PCR analysis of samples was carried using SCAR marker flanking gene Yr10 at distance 0.5 cM. The expected amplification fragment size for locus SCAR Yr10 is 200 bp, while for the susceptible allele of the gene that size is 180 bp[32]. Figure 1 presents DNA electrophoretogram of the DNA amplification product. Isogenic line Yr10/6*AvocetS was used as a positive control. PCR products of size 200 bp, specific for carriers of gene Yr10 were detected in 7 wheat samples including Moro, Karasai, Mereke-70, Mereke-75, Sultan-2, Naz and Akdan.

For identification of Yr18/Lr34 gene carriers, PCR amplification was performed using a pair of primers csLV34-F and R. STS - marker csLV34 flanks the locus Yr18, the genetic distance between loci csLV34 and Yr18 corresponds to 0.4 cM. Locus csLV34 is in the proximal position relative to Yr18 gene. It is known that Yr18 is a "slow rusting gene", which provides long-term and non-specific resistance of the adult plant. This efficient gene is located on the short arm of chromosome 7D[33]. Molecular screening was done using a specific co-dominant STS marker csLV34, which is a bi-allelic locus. Codominant amplification products, denoted as "a" or "b" allele of locus csLV34, are

Table 1. List of available yellow rust markers gene

Gene	Marker type	Primer sequence (5'-3')	Fragment size (bp)	Reference
Yr10	*Yr10SCAR*	CTG CAG AGT GAC ATC ATA CA TCG AAC TAG TAG ATG CTG GC	200/180 bp	*Shao et al. 2001*
Yr15	*SSRXgwm11*	GTG AAT TGT GTC TTG TAT GCT TCC GGA TAG TCA GAC AAT TCT TGT G	215/200 bp	*Murphy et al.* 2009
Yr15	*SSR Xbarc8*	GCG GGA ATC ATG CAT AGG AAA ACA GAA GCG GGG GCG AAA CAT ACA CAT AAA AACA	221/257 bp	*http://maswheat*
Yr18 /Lr34	*STScsLV34*	GTT GGT TAA GAC TGG TGA TGG TGC TTG CTA TTG CTG AAT AGT	150/229 bp	*Lagudah et al.* 2006

Table 2. Phytopathologic evaluation and molecular screening of wheat varieties in Kazakhstan

Samples	Resistance to yellow rust, natural background			Molecular screening of Yr genes			
	1-count	2-count	3-count	Yr10 SCAR	Yr15 Gwm11	Yr15 xbarc8	Yr18 csLv34
Arap	15MS	30S	40S	-	-	-	+
Aliya	0	0	0	-	-	-	-
Almaly	0	20MS	50S	-	-	-	+
Azharly	0	5R	5R	-	-	-	-
Alikhan	0	0	0	-	-	-	-
Akdan	0	0	0	+	-	-	-
Egemen	0	0	0	-	-	-	-
Egemen 20	0	0	20MS	-	-	-	-
Karlygash	30MS	30S	60s	-	-	-	-
Keremet	5MR	30MS	40MS	-	-	-	-
Karasai	5MR	20MS	40S	+	-	-	+
Krasnovodo-padskaya	10MS	20S	40S	-	-	-	-
Kizyl-biday	0	0	10MS	-	-	-	-
Maira	0	0	0	-	-	-	-
Mereke-70	5MR	15MS	20MS	+	-	-	+
Mereke-75	10MR	10MR	10MR	+	-	-	-
Manshuk	0	0	0	-	-	-	-
Matai	15MS	20MS	20MS	-	-	-	-
Naz	0	20MS	30MS	+	-	-	-
Nureke	0	15MS	30MS	-	-	-	+
Dastan	0	20MS	30MS	-	-	-	-
Dinara	0	20MS	30MS	-	-	-	+
President	0	20MS	40S	-	-	-	-
Raminal	0	0	15MS	-	+	+	-
Sultan-2	0	R	5R	+	-	-	-
Intensivnaya	0	0	5R	-	-	-	-
Tungish	0	0	0	-	-	-	-
Taza	10MS	20MS	50S	-	-	-	-
Zhetisu	0	0	15MS	-	-	-	-
Kazakhstan10	0	0	0	-	-	-	-
Morocco (control)	40MS	60S	100S	-	-	-	-
Yr10 (control)	0	5R	15MR	+	-	-	-
Moro (control)	0	0	0	+	-	-	-
Yr 15 (control)	0	0	0	-	+	-	-

Table 3. Agronomic performances of Kazakhstan varieties-carriers of YR10 and YR15 gens

Varieties	Plant height, cm	Head length,cm	Number of spikes in head	Number of grains in main head	Mass of grains in main head, g	Mass of 1000 grains, g
Akdan	94	7,40	18,00	46,40	2,11	45,67
Karasai	112	10,42	20,40	51,10	2,41	47,02
Mereke-70	78	8,45	18,20	40,40	1,90	47,10
Mereke-75	73	10,59	19,30	45,50	1,88	41,34
Naz	71	9,61	19,20	48,00	1,69	35,41
Raminal	91	9,60	21,00	48,70	2,53	51,90
Sultan-2	87	16,09	27,80	90,60	3,29	56,15
Almaly (control)	99	9,71	20,20	43,6	2,18	50,02

homozygous susceptible or sustainable Yr18 allele of the gene, respectively. Figure 2 shows the position of the allele of csLV34 locus. The susceptible allele "a" of Yr18 gene locus generates amplification product with size 229 bp, resistant allele "b" - a product of size 150 bp.

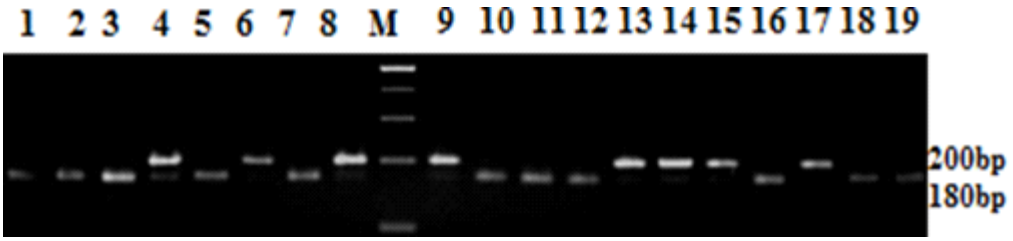
The research results showed that five wheat varieties (Mereke-70, Almaly, Arap, Karasai, Dinara) are carriers of gen complex *Yr18/Lr34.*

Dominant gene Yr15 was transferred to wheat from the G-25 sample of *Triticum dicoccoides Korn*. and localized on the short arm of chromosome 1B[34.35]. The source of Yr15 is Dippes Triumph, and the testing line is *T.diciccoides G-25*. X. Chen's researches have not revealed the North American isolates virulent to Yr15[36]. Our previous studies demonstrated that this gene is also effective against yellow rust isolates in Kazakhstan[9].

In order to identify the Yr15 gene carriers, PCR analysis of wheat genotypes was performed using SSR marker Xgwm11, located at a distance of 6.2 cM from the desired gene. The expected amplification fragment size for locus Xgwm11, coupled with the R-allele of Yr15 gene is 215 bp, and for one coupled with an S-allele is 213 bp[5]. The wheat samples which showed resistance to local population of stripe rust on infectious background (5R-10MR) were analyzed. The DNA fragments that indicate the presence of Yr15 gene were found in wheat variety Raminal and isogenic line Yr15 / 6 * Avocet S. (Figure 3).

To identify the carriers of Yr15 gene, PCR analysis of wheat samples was performed using xbarc8 marker. The expected amplification fragment size of locus xbarc8 linked to the resistant allele of gene Yr15 is 221 bp, while for the locus linked with susceptible allele of the gene the size is 257 bp. PCR product of size of 221 bp which is specific for carriers of Yr15 gene was detected in 2 samples: variety Raminal and isogenic line *Yr15/6* Avocet S*. The remaining wheat samples showed a band of size 257 bp (Figure 4)

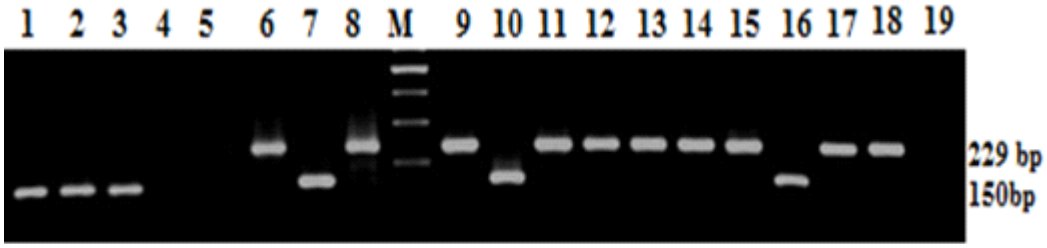
Agronomic performances (plants height, head length, number of spikes in the head, number of grains in the main head, mass of grains in the



1 – Almaly, 2 – Arap, 3 – Nureke, 4 – Akdan, 5 – Dinara, 6 – Karasai, 7 – Morocco (Negative control), 8 – Moro, M-molecular weight marker (Gene Ruler 100 bp DNA Ladder), 9 – Yr10 (positive control, ïî÷òè èçîãåííàÿ ëèíèÿ Yr10/ 6*Avocet S), 10 – Raminal, 11 – Aliya, 12 – Alikhan, 13 – Mereke-70, 14 – Mereke-75, 15 – Naz, 16 – Egemen, 17 – Sultan-2, 18 – Maira, 19 – Manshuk.

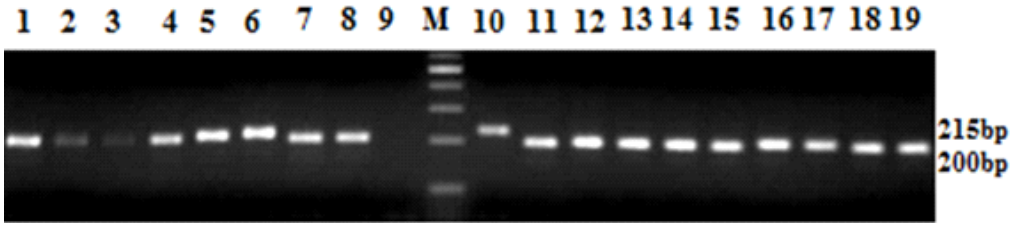
Picture 1. Products of DNA amplification of wheat samples using primers to the locus linked to resistance gene Yr10

main head, mass of 1000 grains) of Kazakhstan wheat varieties – carriers of gens of resistance to yellow rust were investigated by means of molecular screening. In results (Table 3), variety Karasai stands out for plant height (112cm) in comparison with other varieties. Highest values in head length were shown by varieties Karasai (10,42 cm) Mereke-75 (10.59 cm) and Sultan-2 (16.09 cm), the biggest number of spikes in the head was shown by the same varieties and by Raminal, values were within 20,40 cm and 27,80 cm. Number of grains in the main head was high in all the varieties except Mereke-70. Highest values in mass of grains in the main head and mass of 1000 grains were



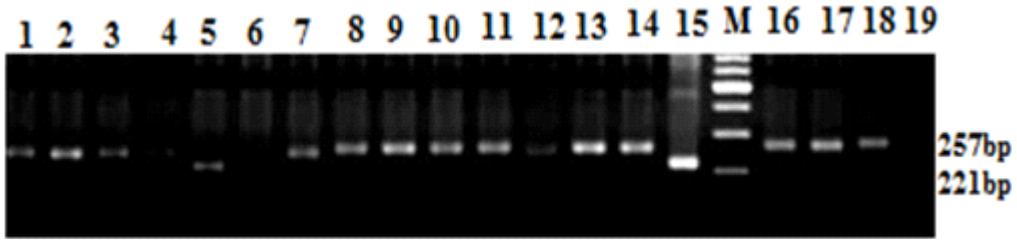
1 – Almaly, 2 – Arap, 3 – Nureke, 4 – Akdan, 5 – Egemen, 6 - Matai, 7 – Karasai, 8 – Raminal, M- molecular weight marker (Gene Ruler 100 bp DNA Ladder), 9 - Morocco (Negative control), 10 – Yr18 (positive control, almost isogenic line Yr10/6*Avocet S), 11 – Aliya, 12 – Alikhan, 13 – Mereke-70, 14 – Mereke-75, 15 – Naz, 16 – Dinara, 17 – Sultan-2, 18 – Maira, 19 – Manshuk.

Picture 2. Molecular screening of Kazakhstan varieties of wheat using primers of locus csLV34



1 – Almaly, 2 – Arap, 3 – Nureke, 4 – Akdan, 5 –Egemen, 6 - Raminal, 7 – Karasai, 8 – Matai, 9 - Negative control (ddH_2O), M- molecular weight marker (Gene Ruler 100 bp DNA Ladder), 10 – Yr15 (positive control, almost isogenic Yr15/6*Avocet S), 11 – Aliya, 12 – Alikhan, 13 – Mereke-70, 14 – Mereke-75, 15 – Naz, 16 – Dinara, 17 – Sultan-2, 18 – Maira, 19 – Manshuk.

Picture 3. Identification of gen Yr15 using marker SSRXgwm11



1 – Almaly, 2 – Arap, 3 – Nureke, 4 – Akdan, 5 – Raminal, 6 – Egemen, 7 – Karasai, 8 – Matai, 9 – Naz, 10 – Mereke-75, 11 – Aliya, 12 – Alikhan, 13 – Mereke-70, 14 – Morocco (Negative control), 15 – Yr15 (positive control, Yr15/6*Avocet S), M- molecular weight marker (Gene Ruler 100 bp DNA Ladder), 16 – Dinara, 17 – Sultan-2, 18 – Maira, 19 – Manshuk.

Picture 4. Identification of Yr15 gen using marker Xbarc8

demonstrated by Raminal and Sultan-2.

All in all, the structural analysis demonstrated that varieties Karasai, Raminal, Sultan-2 have higher performances by several factors in comparison with standard variety Almaly.

CONCLUSION

Several varieties were selected based on results phytopathologic evaluation of susceptibility to wheat yellow rust *(Puccinia striiformis)*. Within 30 investigated native varieties, Alikhan, Egemen, Akdan, Maira, Manshuk, Tungush, Kazakhstan-10 were 0-immune ones, without infection symptoms.

Carriers of effective gens of resistance to wheat yellow rust were identified by means of using molecular markers linked to Yr-resistant gens. Based on the results of molecular screening and phytopathologic evaluation, varieties with Yr10 genom – Karasai, Moro, Naz, Mereke-70, Mereke-75, Sultan-2, Akdan and only variety Raminal with genom Yr15 were identified. Varieties Karasai and Mereke-70 are the most valuable donors of resistance to yellow rust.Two gens of resistance to yellow rust were found in them.

Varieties Karasai, Raminal, Sultan-2 demonstrated higher performances of some productivity elements in comparison with standard variety Almaly in the structural analysis.

In order to accelerate the selection process, we will continue selection of lines resistant to diseases by means of using molecular markers linked to this mark. It becomes possible to use these identified varieties with Yr gens in perspective as donors. Due to molecular-genetic methods and MAS technology, the results of our work Ìîëåêóëàðû-ãåíåòèêàëû Ùä³ñòòåðä³ æÙíå Marker Assisted Selection òåõíîëîãèÿñûí ›îëäàíó àð›àñûíäà, á³çä³£ allowed to get a new level in selection processes in Kazakhstan.

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