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MOLECULAR SNP MARKING OF SPRING WHEAT BY *TADR1* GENE ON THE DROUGHT RESISTANCE

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ABSTRACT

Under the conditions of climate change, the problem of drought tolerance of agricultural crops has an increasing effect. Therefore, an important direction of modern breeding, and today, is to improve the drought tolerance of plants when creating new varieties. To assess the resistance of plants, various methods are used, including laboratory ones. In physiological evaluation, an effective method is to assess drought tolerance at the seedling stage. A deeper analysis is molecular identification based on the genes that control the trait under study. The article presents the results of studies on the assessment of soft durum wheat samples for resistance to osmotic stress and their molecular screening using marker technology. The purpose of the research is to study and choice the selected varieties of spring wheat on the basis of drought tolerance. As a result of molecular screening for the *TaDr1* gene for drought resistance were identified cultivars and lines of soft spring wheat: Dynasty, Stepnaya 53, Stepnaya 75, Lutescens 268, Astana, Omskaya 38, Saratovskaya 42, Glenlea, Novosibirskaya 29, Shortandinskaya 2007, Akmola 2, Duet, Saratovskaya 55, Chebarkulskaya; Shortandinskaya 95 ul., Lin. 201/21; Lin. 198/224-21; 176/09; 233/10; 312/10; 342/08; 248/10; 329/11; 4/13; 55/08; 225/12; 129/12; 371/13; 238/09; Lutescens 2203; 659/12; 486/lyut 22; 63/ lyut 37; 23/07; spring durum wheat: Omskaya yantarnaya, line 69-08-2; 23/07.

Key words: wheat, drought, resistance, gene, SNP marker, genotyping.

INTRODUCTION

The disease is a recognized and devastating abiotic stress for crops, especially in the increased frequency of plant development, detrimental to plant yield throughout the history of agriculture [1]. Drought damage is significant damage from any other stressor [2]. In recent years, the efforts of many foreign and domestic scientists have been aimed at studying the nature of drought resistance of wheat plants (*Triticum aestivum L.*) [3, 4]. Currently, the main goal of breeders is to create genotypes of agricultural plants capable of withstanding the moisture deficiency in the critical phases of development for them, combined with a high yield potential [5].

Direct assessment of drought resistance in the field, with all its objectivity, requires long-term observations. Drought does not happen every year, and its character also changes. To speed up the breeding process, more often resort to indirect assessment of drought resistance using laboratory physiological methods.

Determining the stress resistance of plants in the field is possible only under the influence of extreme environmental conditions, and many researchers use laboratory express diagnostics. However, the assessment of drought resistance in laboratory conditions is only a confirmation of field experiments, since plants in the seedling phase are most sensitive to stress, and the differences that appear between varieties during this period are preserved as a genetic trait in adult plants [6, 7]. The literature presents a large number of methods for diagnosing resistance to stress factors, both in the field and in the laboratory [8]. Methods of early diagnostics on seeds and seedlings are of particular interest, since they allow assessment all year round and analysis of a large amount of breeding material [9].

With the development of modern science, assessment at the gene level is used to obtain stress-resistant plants, the use of molecular biology methods may become the dominant breeding tool [10]. The study of selected genotypes using molecular genetic markers is widely used in various breeding programs. At the same time, the main stage of planning in breeding is the selection of a target gene, which, in response to stress inside plant cells, actively turns on various regulatory systems, including drought resistance.

The transcription factors *TaNFY,* Nuclear Factor *Y (NF-Y))*, *TaDREB* (Dehydration-responsive element binding), *TaDr1* (Down-regulator) are effective genes in wheat breeding in case of drought of transcription factors. These genes are expressed differently in high- and low-yielding wheat varieties in response to the duration of drought and dehydration. Highly expressed transcription factors are associated with better rooting, nitrogen uptake, filling, and both show increased drought tolerance and yield [11-15].

The transcriptional repressor *TaDr1* is involved in the control of expression of the *TaVrn1* vernalization and *TaFT1* flowering genes. Increased expression of yield increase genes in wheat varieties phenological changes that positively affect the susceptibility and productivity of plants in arid conditions to phenological changes that affect plant productivity and, therefore, explain differences in the adaptation of the choice of wheat varieties to arid conditions [11]

Thus, in order to create resistant varieties for arid climates, a necessary measure is the use of physiological and molecular genetic assessment for more accurate and in-depth analysis when studying complex traits such as drought resistance.

In connection with the foregoing, the purpose of the research is to select the identified varieties of spring wheat on the basis of drought tolerance, through laboratory tests.

MATERIALS AND RESEARCH METHODS

The studies were carried out on the basis of the «Research platform of agricultural biotechnology» at the NJSC «S. Seifullin Kazakh Agrotechnical University». Research material - 58 varieties of spring wheat of various ecological and geographical origin, represented by the leading breeding centers

of Northern, Central and Western Kazakhstan (Figure 1). Including breeding lines of soft and durum wheat. As a positive (carrier of polymorphism) and a negative control during genotyping, DNA of 2 varieties of *Triticum spelta (18-4)* and [(*T. aestivum cv*. Novosibirskaya 67 / *T. dicoccum*)] (18-5) were involved in the experiment.

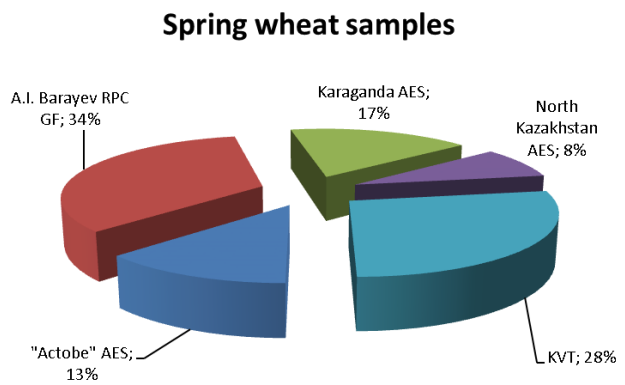

Figure 1 - Spring wheat samples for assessing relative drought tolerance

The laboratory work consisted of two stages: at stage I, for the physiological assessment of drought resistance, the ability of wheat seeds to germinate in a sucrose solution was determined; at stage II, molecular genetic screening of varieties and breeding lines of wheat was carried out using the SNP marker KATU-W62, developed for the *TaDr1* gene, which is closely associated with the trait of drought resistance in wheat.

For laboratory work on seed germination on sucrose solutions with high osmotic pressure, the method of Oleinikova T.V. [16] was used. Germination of seeds was carried out on filter paper in Petri dishes, previously sterilized for 2 hours at a temperature of 70^{0}C. Healthy seeds of spring wheat samples were selected for the experiment. Seeds were germinated at concentrations of sucrose solution with osmotic pressure: for soft wheat - 16 atm., for durum wheat - 10 atm. The repetition is 4-time, the sample size is 25 seeds for each variety. Control - distilled water. After 7 days, the percentage of germinated seeds was determined by calculating their average number (*a*) in sucrose solution from the number of seeds germinated in the control (*b*), that is, $P = (a/b) \times 100\%$. The highest percentage of seed germination in sucrose solution indicated the drought resistance of the sample.

In a molecular genetic study, selection was made for the *TaDr1* gene, a transcription repressor whose function is to inhibit the expression of other genes and is closely associated with wheat drought resistance [11]. Genotyping of the studied wheat samples was carried out according to the published method of M.V. Myakishev [17] using the KATU-W62 marker developed for this gene and tested Amplifluor-like SNP [11]. DNA extraction was carried out by the CTAB method [18]. Each reaction contained a Master-Mix: PCR buffer, $MgCl_2$ (50 mM), 2,5 µM of each fluorescent label of the universal probe, dNTP (2 mM), each of the two forward primers (1.5 µM), and 7.8 µM reverse complement primer, and 0.1 units of SibEnzyme DNA polymerase (Russia, Novosibirsk) with the addition of 3 µl of ROX passive reference ROX (ThermoFisher, USA). The master mix The master mix was poured into 96-well and 384-well microplates. Genomic DNA (20 ng/µl) was added to each well, 2 µl. PCR was performed using the program according to the published protocol of Rickert A.M., with modifications [19], on a quantitative PCR instrument 'QuantStudio7 Flex Real-Time PCR System' from 'ThermoFisher-ABI'.

For genotyping, a short program and lower annealing temperatures for the KATU-W62 primer were used. Initial denaturation at 94°C, 2 min; 14 «doubled» cycles at 95°C for 10 s, 56°C for 2 min, 94°C for 15 s, 50°C for 2 min and 62°C for 5 min.

RESULTS

According to the diagnostic results, in the experiment, variety samples and breeding lines were distributed into drought resistance groups. Among the «highly resistant» samples were those with germination on sucrose solution > 50%, they respectively constituted group I. The second group of «medium-resistant» was characterized by 30-50% germination in sucrose solution. The third group consisted of «weakly resistant» samples with a germination rate below 30%.

As a result of the screening, the best results in terms of drought resistance were shown by which varieties germination on a sucrose solution to the control (distilled water) was 100% (Figure 2).

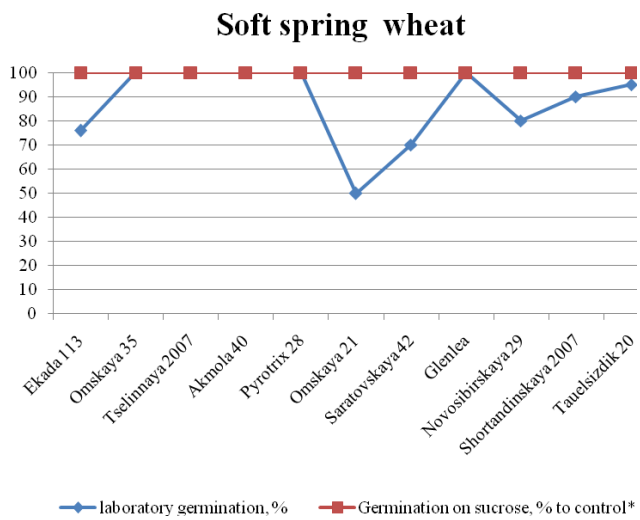

Figure 2 - Evaluation of soft wheat accessions for drought resistance at early stages of seedling development, % of control

According to laboratoru recearch, among the varieties of soft spring wheat, the highly resistant to drought conditions at an early stage of seedling development were varieties of soft spring wheat Ekada 113 (Aktobe AES), Tselinnaya 2007, Akmola 40, Shortandinskaya 2007, Tauelsizdik 20 (A.I. Barayev RPC GF), as well as varieties of foreign selection Omskaya 35, Pyrotrix 28, Omskaya 21, Saratovskaya 42, Novosibirskaya 29, Glenlea.

Among durum wheat varieties, the "highly resistant" varieties include varieties Omskaya yantarnaya, Korona, Damsinskaya 90 (Figure 3), lines 188-05-4, 225-09-5, 69-08-3, 148-11-11, 272 -08-9, 69-08-2 (Table 1).

Among the «highly resistant» breeding lines include: Lin. P-1415m, Lin. 225/21 (Aktobe AES), 248/10, 4/13, 330/12,

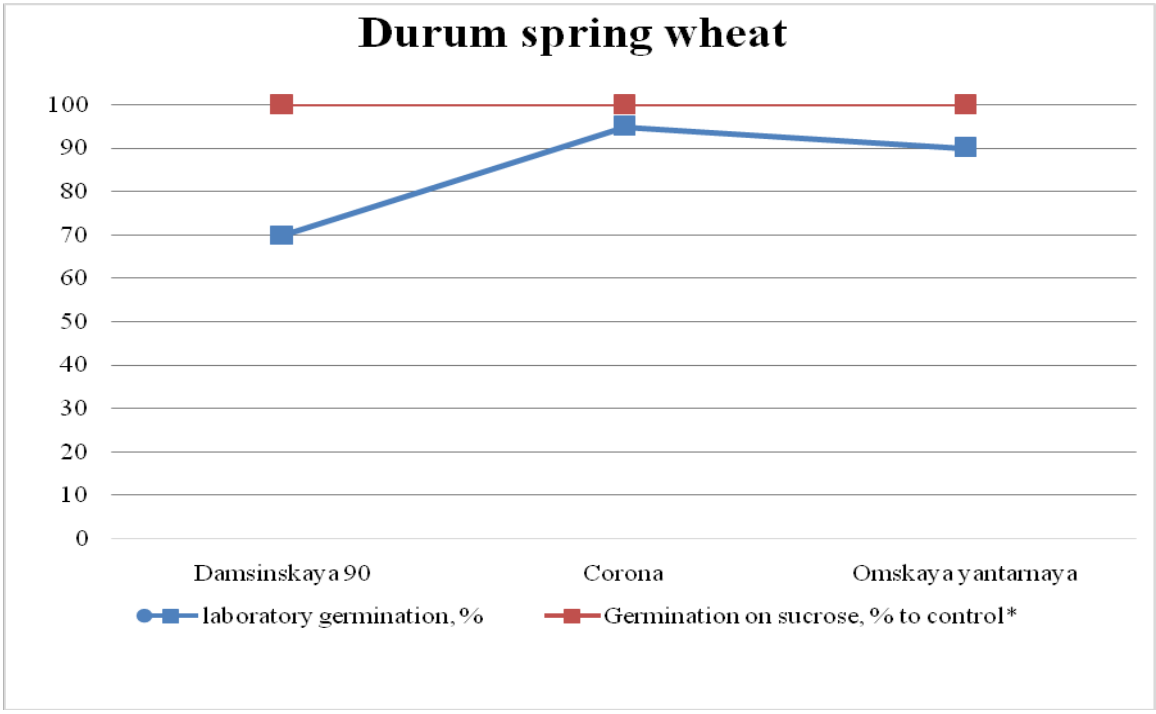

Figure 3 - Evaluation of durum wheat samples for drought resistance at early stages of seedling development, % of control

Table 1 - Diagnosis of spring wheat lines at the early stages of seedling development for drought resistance

№	Variety, line	Laboratory germination, %	Germination on sucrose, % to control*	Group of drought tolerance
Soft spring wheat				
1	Lin. P-1415м	64	57,8	I
2	Lin. 225 / 21г.	78	75,6	I
3	248/10	89	52,8	I
4	4/13	96	54,2	I
5	330/12	74	77,0	I
6	225/12	84	61,9	I
7	66/10	98	62,2	I
8	Az x K-55	96	58,2	I
9	Az x K-191	92	50,6	I
10	KxAzh-177	90	51,1	I
Spring durum wheat				
1	188-05-4	77	67,5	I
2	69-08-2	86	61,6	I
3	69-08-3	90	55,5	I
4	148-11-11	88	65,9	I
5	272-08-9	73	58,9	I
6	225-09-5	77	72,7	I

* - germination on a solution of sucrose 16 atm. for soft wheat, 10 atm. for durum wheat, % to germination on control (distilled water)

Table 2 - Sequence of oligonucleotides for genotyping for drought resistance

Primer name	Primer sequence
KATU-W62-SNP2 -F5S	GAAGGTGACCAAGTTCATGCTAGTGGTGTCCTCCTTCTAATTT
KATU-W62-SNP2-F5D	GAAGGTCGGAGTCAACGGATTAGTGGTGTCCTCCTTCTAATTA
KATU-W62-SNP2-R	TCATAGGGCGAGTGTGCATA

225/12, 66/10 (A.I. Barayev RPC GF), Az x K-55, Az x K-191, KxAzh- 177 (S. Seifullin KATU), (Table 1).

Selection based on the study of the genotype allows for more efficient selection. At the second stage, wheat samples were screened for drought resistance using their own, previously developed molecular markers. When genotyping wheat varieties and breeding lines, 'Amplifluor-like' SNP molecular markers were used, one of which is W62SNP2, developed on the basis of the *TaDr1* gene that controls plant response to drought (Table 2) [11]. The best genotypes for the *TaDr1* transcription repressor gene are soft spring wheat varieties: Dynasty, Stepnaya 53, Stepnaya 75, Lutescens 268, Astana, Omskaya 38, Saratovskaya 42, Glenlea, Novosibirskaya 29, Shortandinskaya 2007, Akmola 2, Duet, Saratovskaya 55, Chebarkulskaya; Shortandinskaya 95 ul.

Among durum wheat varieties, the variety Omskaya yan-

tarnaya stands out, and lines 69-08-2; 23/07 selection A.I. Barayev RPC GF, among the lines of soft spring wheat according to the results of two technical repetitions: Lin. 201/21; Lin. 198/224-21 (Aktobe AES); 176/09; 233/10; 312/10; 342/08; 248/10; 329/11; 4/13; 55/08; 225/12; 129/12; 371/13; 238/09 (KVT); Lutescens 2203 (Karaganda AES); 659/12; 486/lyut 22; 63/ lyut 37; 23/07 (Northern Kaz. AES).

DISCUSSION

Wheat improvement in order to enrich domestic wheat genotypes with wild forms is an effective way to obtain a good source material. The Amplifluor-like' SNP molecular marker KATU-W62 was created by working with a complex 18-6 interspecific hybrid between spelt, soft wheat and a wild relative: *18-4♀ Triticum spelta × 18-5 ♂* [(*T. aestivum cv. Novosibirskaya 67 / T. dicoccum*)]. Hybrids of spring soft wheat, obtained by crossing with wild relatives, are sources of economically valuable traits.

Genetic polymorphism was found in the B genome of the paternal parent 18-5, and when genotyping with this primer, the 18-5 sample was identified as the *'aa'* allele, as well as higher-yielding hybrids under drought conditions [11]. Based on the above, genotypes 18-4, 18-5 were introduced into the experiment as identifiers when optimizing the genotyping process of spring wheat accessions.

As a result of the studies, the genotypes of varieties and lines clearly differed from each other by the KATU-W62 primer and corresponded to one of three options: coinciding with the 18-4 genotype *'bb'*, with the high-yielding genotype 18-5 *'aa'* in drought conditions, or were heterozygotes for both *'ab'* alleles.

Relative fluorescence units (RFU) for the FAM and VIC dyes are shown on the X- and Y-axes of the distribution, respectively. Red circles denote homozygotes *'aa'*; blue – homozygotes *'bb'*; green - heterozygotes *'ab'* or a mixture of genotypes; black crosses indicate unidentified genotypes, black squares NTC (water) (Figure 4).

Figure 4 shows that 38 varieties of spring wheat, indicated by red circles, were identified as homozygous for the allele *'aa'*, along with sample 18-5, a polymorphism carrier. The samples separated by the fluorophore FAM, in both technical repetitions, were selected as the best genotypes for the gene *TaDr1* repressor transcription. Among such genotypes, among the varieties and hybrid lines W1-W117, there are varieties of spring soft wheat: Dynasty (W6-2021), Stepnaya 53 (W7-2021), Stepnaya 75 (W8-2021), Lutescens 268 (W17-2021), Astana (W26-2021), Omskaya 38(W27-2021), Saratovskaya 42(W31-2021), Glenlea(W32-2021), Novosibirskaya 29 (W35-2021), Shortandinskaya 2007(W36-2021), Akmola 2(W37- 2021), Duet (W38-2021), Saratovskaya 55 (W40-2021), Chebarkulskaya (W41-2021); Shortandinskaya 95 ul. (W107-2021). Among the varieties of durum wheat, the variety Omskaya yantarnay (W44-2021), line 69-08-2 (W68-2021); 23/07 (W101-2021) selection of A.I. Barayev RPC GF, among the lines of spring soft wheat after the results of two technical repetitions: Lin. 201/21. (W10-2021); Lin. 198/224-21g. (W14-2021); 176/09 (W47-2021); 233/10(W48-2021); 312/10 (W50-2021); 342/08 (W52-2021); 248/10 (W53-2021); 329/11(W54-2021); 4/13(W55); 55/08(W56-2021); 225/12 (W60-2021); 129/12 (W62-2021); 371/13(W65-2021); 238/09(W66-2021); Lutescens 2203 (W82-2021); 659/12(W98-2021); 486/lute 22 (W99-2021); 63/ lute 37(W100-2021); 23/07 (W101-2021). In turn, the samples separated as homozygotes for the allele *'bb'* are recognized as carriers of the polymorphism, on the basis of which the primer KATU -62 was developed for the *TaDr1* gene. Such samples include varieties Zhemchuzhina Sibiri, Amethyst, Robblin and selection lines Lin 225/21, Hybrid 4280-10, 148-11-11, 219-09-7. And 11 lines, indicated by a green circle in the figure, are heterozygous (ab) for this gene or represent a mixture of seeds. Such samples include Lutescens 728, Lutescens 2222,

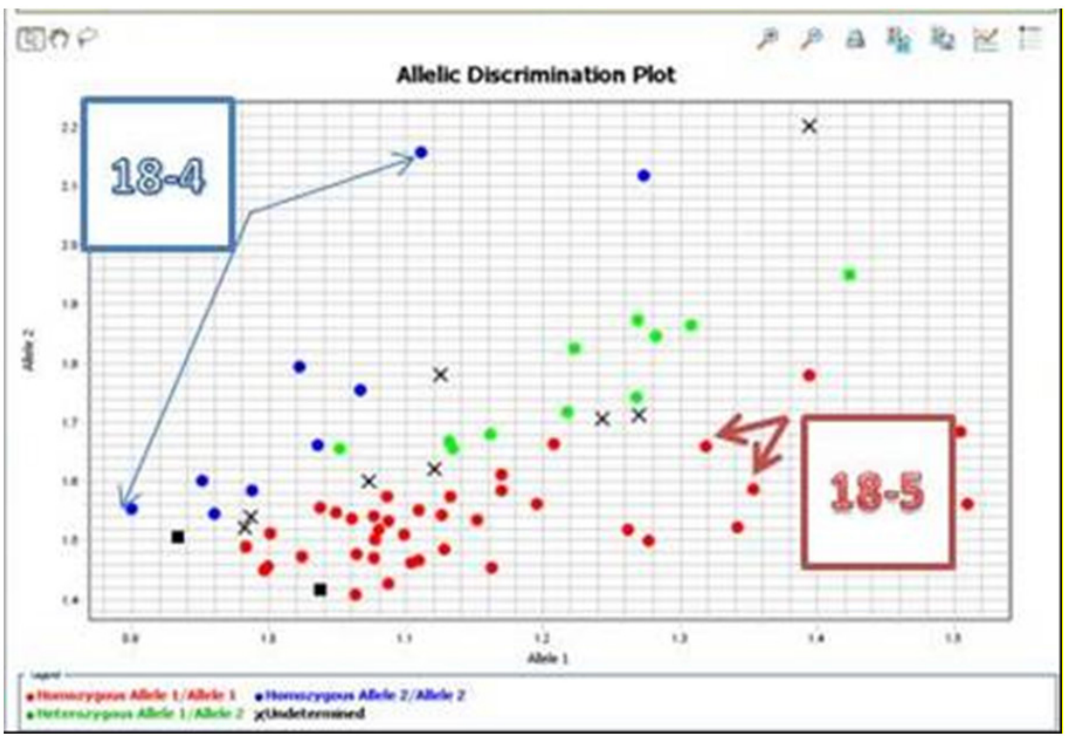

Figure 4 - Genotyping using the molecular Amplifluor-like SNP marker KATU-W62-SNP2, developed for the *TaDr1* gene

Lutescens 2202, Lutescens 1519, Lutescens 2262, 320/10, 27/11, Lute 13/12, Erit 42/12, 225-09-5, 66/10.

CONCLUSION

According to the results of the experiment, among the spring soft wheat varieties, the highly resistant to drought conditions at the early stage of seedling development were varieties of spring soft wheat Ekada 113 (Aktobe AES), Tselinnaya 2007, Akmola 40, Shortandinskaya 2007, Tauelsizdik 20 (A.I. Barayev RPC GF), as well as varieties of foreign selection Omskaya 35, Pyrotrix 28, Omskaya 21, Saratovskaya 42, Novosibirskaya 29, Glenlea. Among the «highly resistant» breeding lines include: Lin. P-1415m, Lin. 225/21 (Aktobe AES), 248/10, 4/13, 330/12, 225/12, 66/10 (A.I. Barayev RPC GF), Lutescens 2264, Lutescens 2231 (Karaganda AES), Az x K- 55, Az x K-191, KxAzh-177 (S. Seifullin KATU). Among durum wheat varieties, as «highly resistant» varieties are Omskaya yantarnaya, Korona, Damsinskaya 90, lines 188-05-4, 225-09-5, 69-08-3, 148-11-11, 272-08-9, 69-08-2.

As a result of genotyping of spring wheat cultivars for the *TaDr1* gene using the primer KATU W-62, varieties of spring common wheat were identified: Dynasty, Stepnaya 53, Stepnaya 75, Lutescens 268, Astana, Omskaya 38, Saratovskaya 42, Glenlea, Novosibirskaya 29, Shortandinskaya 2007, Akmola 2, Duet, Saratovskaya 55, Chebarkulskaya, Shortandinskaya 95 ul. The following samples of spring durum wheat should also be noted: Omskaya yantarnaya, lines 69-08-2; 23/07 selection A.I. Barayev RPC GF. The breeding lines identified by the results of two technical repetitions are also identified as donors *TaDr1* gene, among them the following showed the reliable result during genotyping: lines of soft wheat Lin. 201/21; Lin. 198/224-21; 176/09; 233/10; 312/10; 342/08; 248/10; 329/11; 4/13; 55/08; 225/12; 129/12; 371/13; 238/09; Lutescens 2203; 659/12; 486/ lyut 22; 63/ lyut 37; 23/07. As a result of studies of soft wheat varieties Shortandinskaya 2007, Saratovskaya 42, Novosibirskaya 29, durum wheat Omskaya yantarnaya, as well as lines 225/12, 248/10, 4/13 of the A.I. Barayev RPC GF were the best in the two given in article on methods for assessing drought resistance, both at an early stage of seedling development under the influence of sucrose, and during genotyping with the KATU W-62 primer designed for the *TaDr1* gene. The cultivars Zhemchuzhina Sibiri, Ametist, Robblin and the breeding lines Lin 225/21, Hybrid 4280-10, 148-11-11, 219-09-7 do not carry the polymorphism on the basis of which the KATU-62 primer for the *TaDr1* gene was developed. And the lines Lutescens 728, Lutescens 2222, Lutescens 2202, Lutescens 1519, Lutescens 2262, 320/10, 27/11, Lyut 13/12, Erith 42/12, 225-09-5, 66/10 are determined to be heterozygous (*ab*) for the desired transcription repressor *TaDr1* gene or a mixture of seeds of different genotypes.

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МОЛЕКУЛЯРНОЕ SNP – МАРКИРОВАНИЕ ЯРОВОЙ ПШЕНИЦЫ ПО ГЕНУ *TADR1* НА ЗАСУХОУСТОЙЧИВОСТЬ

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АННОТАЦИЯ

В условиях изменения климата, проблема засухоустойчивости сельскохозяйственных культур приобретает нарастающий эффект. Поэтому важным направлением современной селекции, и сегодня, - является повышение засухоустойчивости растений при создании новых сортов. Для оценки устойчивости растений применяют различные методы, в том числе и лабораторные. При физиологической оценке, эффективным методом является оценка засухоустойчивости на стадии проростка. Более глубоким анализом является молекулярное маркирование на основе генов, контролирующих исследуемый признак. В статье представлены результаты исследований по оценке образцов мягкой и твердой пшеницы на устойчивость к осмотическому стрессу, и их молекулярный скрининг по маркерной технологии. Цель исследований – изучить и провести отбор выделившихся сортообразцов яровой пшеницы по признаку засухоустойчивости. В результате молекулярного скрининга по гену *TaDr1* на засухоустойчивость выделены сорта и линии яровой мягкой пшеницы: Династия, Степная 53, Степная 75, Лютесценс 268, Астана, Омская 38, Саратовская 42, Glenlea, Новосибирская 29, Шортандинская 2007, Акмола 2, Дуэт, Саратовская 55, Чебаркульская; Шортандинская 95 улучшенная, Лин. 201/21г.; Лин. 198/224-21г.; 176/09; 233/10; 312/10; 342/08; 248/10; 329/11; 4/13; 55/08; 225/12; 129/12; 371/13; 238/09; Лютесценс 2203; 659/12; 486/лют 22; 63/ лют 37; 23/07.; яровой твердой пшеницы: Омская янтарная, линии 69-08-2; 23/07.

Ключевые слова: пшеница, засуха, устойчивость, ген, SNP маркер, генотипирование.

ЖАЗДЫҚ БИДАЙДЫ *TADR1* ГЕНІ БОЙЫНША ҚҰРҒАҚШЫЛЫҚҚА ТӨЗІМДІЛІГІН МОЛЕКУЛЯРЛЫҚ МАРКЕРЛЕУ

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ТҮЙІН

Климаттың өзгеруі жағдайында ауылшаруашылық дақылдарының құрғақшылыққа төзімділігі мәселесі күшейіп келеді. Сондықтан қазіргі заманғы селекцияның маңызды бағыты және бүгінгі күні жаңа сорттарды жасау кезінде өсімдіктердің құрғақшылыққа төзімділігін арттыру болып табылады. Өсімдіктердің төзімділігін бағалау үшін әртүрлі әдістер, соның ішінде зертханалық әдістер қолданылады. Физиологиялық бағалауда тиімді әдіс - көшеттер кезеңінде құрғақшылыққа төзімділікті бағалау. Тереңірек талдау зерттелетін белгіні басқаратын гендерге негізделген молекулалық таңбалау болып табылады. Мақалада жұмсақ қатты бидай үлгілерінің осмостық стресске төзімділігін бағалау және маркерлік технологияны қолдану арқылы молекулалық скрининг бойынша зерттеулердің нәтижелері берілген. Зерттеу жұмысының мақсаты – құрғақшылыққа төзімділік негізінде жаздық бидайдың іріктелген сорттарын зерттеу және таңдау. Құрғақшылыққа төзімділік *TaDr1* геніне молекулярлық скрининг нәтижесінде жаздық жұмсақ бидай сорттары мен линиялары анықталды: Династия, Степная 53, Степная 75, Лютесценс 268, Астана, Омская 38, Саратовская 42, Glenlea, Новосибирская 29, Шортандинская 95 жақсартылған, Ақмола 2, Дуэт, Саратовская 55, Чебаркульская; Шортандинская 95 жақсартылған, Лин. 201/21; Лин. 198/224-21; 176/09; 233/10; 312/10; 342/08; 248/10; 329/11; 4/13; 55/08; 225/12; 129/12; 371/13; 238/09; Лютесценс 2203; 659/12; 486/лют 22; 63/ лют 37; 23/07.; жаздық қатты бидай: Омская янтарная, 69-08-2-линиялары; 23/07.

Негізгі сөздер: бидай, құрғақшылық, төзімділік, ген, SNP маркер, генотиптеу.