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IDENTIFICATION AND DISTRIBUTION OF ALLELES OF HYBRID NECROSIS GENE *Ne2* IN SOFT WHEAT CULTIVARS (*TRITICUM AESTIVUM* L.)

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Aim. To determine the correspondence of different alleles of locus *Xbarc55-2B* to alleles of gene *Ne2*, different in their strength, and to investigate the distribution of alleles of locus *Xbarc55-2B* and the corresponding alleles of gene *Ne2* from wheat cultivars of Ukrainian and Russian selection. **Methods.** Polymerase chain reaction, electrophoresis in PAAG, statistical methods. **Results.** The alleles of locus *Xbarc55-2B*, bound to the hybrid necrosis gene *Ne2*, were used to identify the genotypes of 290 soft wheat cultivars of different geographic origin. The correspondence of different alleles of locus *Xbarc55-2B* to alleles of gene *Ne2*, different in their strength, was defined: 142 bp – *ne2*, 136 bp – *Ne2^{w/m}*, 132 bp – *Ne2^{ms}*, 126 bp – *Ne2^s*. The distribution of the identified alleles of gene *Ne2* among the wheat cultivars of Ukrainian and Russian selection was demonstrated. **Conclusions.** The advantage of some alleles for cultivars of different regions was determined, which may testify to their selection and adaptation value.

Keywords: *Triticum aestivum* L., hybrid necrosis gene *Ne2*, microsatellite analysis.

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INTRODUCTION

Hybrid necrosis of wheat is a premature gradual perishing of leaves or plants of wheat F_{1-2} in some combinations of crossing, which is conditioned by the interaction of two dominant complementary genes *Ne1* and *Ne2*, localized in chromosomes 5BL and 2BS, respectively [1].

Hybrid necrosis is a serious obstacle for combining of desired features in one genotype or for the transfer of genes from wild species to commercial cultivars. Also the hybrid necrosis may complicate the outcomes of genetic analysis of some features due to the loss of some genotypes in cleavable populations. Therefore, while selecting parental pairs for crossing, the selection breeders and geneticists should avoid the combination of strong alleles of hybrid necrosis genes. The data about the cultivars, which are carriers of lethal genes *Ne* may be found in the publications [2–6]. However, most cultivars of Ukrainian selection have not been

identified by the alleles of genes of hybrid necrosis. The availability of dominant genes *Ne1* and *Ne2* in cultivars is determined using the classic genetic analysis while crossing with cultivars-testers with genotypes *ne1ne1Ne2^sNe2^s* and *Ne1^sNe1^sne2ne2* [2–6]. This approach is very time- and labour-consuming, and environmental factors impact the phenotype manifestation of hybrid necrosis [7, 8]. The use of molecular markers, closely bound to hybrid necrosis genes, accelerates the identification of genotypes – carriers of genes *Ne1* and *Ne2*. The application of MAS-approach will allow conducting negative selection of genotypes – carriers of strong alleles of hybrid necrosis genes out of valuable selective material or, vice versa, creating commercial cultivars with specific combinations of alleles of the mentioned genes. Chu *et al.* [9] conducted molecular mapping of hybrid necrosis genes *Ne1* and *Ne2* using microsatellite markers and demonstrated that marker *Xbarc74-5B* is bound to *Ne1* at a genetic distance of 2.0 cM, and *Xbarc55-2B* – 3.2 cM from *Ne2*.

The degree of manifestation of hybrid necrosis in hybrids F_1 depends on the strength of interacting dominant alleles: three alleles *Ne1* (*Ne1^w* – weak, *Ne1^m* – medium strength and *Ne1^s* – strong) and five alleles *Ne2* (*Ne2^w* – weak, *Ne2^{mw}* – less than medium, *Ne2^m* – medium strength, *Ne2^{ms}* – above medium strength and *Ne2^s* – strong), differentiated by Hermesen [7] and Zeven [8]. There is no information in scientific literature on the mode of coordination of alleles (amplification products) of loci *Xbarc74-5B* and *Xbarc55-2B* with alleles of genes *Ne1* and *Ne2*, which have different strength.

The aim of this study was to determine the correspondence of different alleles of locus *Xbarc55-2B* to alleles of gene *Ne2*, different in their strength, and to investigate the distribution of alleles of locus *Xbarc55-2B* and the corresponding alleles of gene *Ne2* from wheat cultivars of Ukrainian and Russian selection.

MATERIALS AND METHODS

The study investigated 290 cultivars of soft wheat from the working collection of the Department of general and molecular genetics and the Department of wheat selection and seeds study of the Selection and Genetics Institute – the National Center of Seed and Cultivar Investigation (SGI–NCSCI). 248 cultivars out of all the analyzed genotypes have been created in different selection centers of Ukraine and 42 cultivars originated in the Russian Federation. The cultivars – carriers of known alleles of gene *Ne2*: Chinese Spring (*ne2*), Vakka (*Ne2^w*), Sonalika (*Ne2^m*), Dawson (*Ne2^{ms}*) and Blackhull (*Ne2^s*) were provided by the USDA, Germplasm Resources Information Network (<http://www.ars-grin.gov>).

DNAs were extracted from five-day-old plants using CTAB-buffer [10]. DNAs of five individual plants of each cultivar were investigated. PCR with directed primers for microsatellite locus *Xbarc55-2B* was conducted at the thermocycler “Tertsik” (“DNA-technology”, RF). The reaction mixture of 25 μ l contained the buffer (67 mM tris-HCl, pH 8.4; 16.6 mM $(\text{NH}_4)_2\text{SO}_4$, 2.0 mM MgCl_2 , 0.03 % Tween-20); dNTP in the volume of 0.2 mmol each and 0.25 μ M primer; 40 ng DNA; 1 unit of Taq-polymerase. The layers of 30 μ l mineral oil were applied above the reaction mixture. The reaction conditions: denaturation at 95 °C for 30 s (initial – 5 min), annealing (60 °C, 30 s), elongation (72 °C, 30 min), final elongation (3 min). The amplification products (10 μ l aliquots of PCR-mixture) were fractioned in 12 % non-denaturing polyacryl-

amide gel (PAAG) in buffer $1 \times$ TBE. The electrophoresis in PAAG was conducted at permanent voltage of 500 V in the apparatus for vertical gel-electrophoresis VE-3 “Helicon” (RF). The products of electrophoretic distribution were visualized by impregnating gels with silver nitrate according to Budowle *et al.* [11]. The video-images of amplified fragments were obtained using the videosystem ImageMaster VDS (AmershamPharmaciaBiotech, USA) according to the manual. The molecular mass of the obtained amplicons was calibrated using standard *pUC19/MspI* and 10 bp DNA Ladder.

The statistical processing of the results obtained was conducted using common methods [12].

RESULTS AND DISCUSSION

The amplification of DNA of known cultivars – carriers of different alleles of gene *Ne2* was conducted to determine the correspondence of alleles of microsatellite locus *Xbarc55-2B* to alleles of gene *Ne2*, different in their strength, using directed primers to locus *Xbarc55-2B*: Chinese Spring (*ne2*), Vakka (*Ne2^w*), Sonalika (*Ne2^m*), Dawson (*Ne2^{ms}*) and Blackhull (*Ne2^s*). It was demonstrated that the recessive allele of gene *ne2* corresponds to the allele of locus *Xbarc55-2B* of 142 bp, dominant alleles of gene *Ne2^w* and *Ne2^m* correspond to allele of 136 bp, allele of gene *Ne2^{ms}* – 132 bp and allele of gene *Ne2^s* to allele of 126 bp. (Fig. 1). It was not possible to differentiate cultivars – carriers of alleles *Ne2^w* and *Ne2^m* for the pair of primers by locus *Xbarc55-2*. Allele of 136 bp of locus *Xbarc55-2B* was marked as *Ne2^{w/m}*.

There are literature data on the fact that the resistance gene *Lr13* of adult plants to leaf rust is bound to allele *Ne2^m* [13, 14]. The differentiation of alleles *Ne2^w* and *Ne2^m* would reasonably require available molecular markers to gene *Lr13*. Gene *Lr13* was identified using pairs of primers to microsatellite locus *Xgwm630*, localized at the distance of 10 cM from *Lr13* [15]. The results of the PCR analysis by locus *Xgwm630* of cultivars – carriers of known alleles of gene *Ne2*, as well as isogenic line of Thatcher cultivar with the resistance gene to leaf rust *Lr13* (TcLr13) did not reveal a marker fragment of 123 bp in Sonalika cultivar (*Ne2^m*) (Fig. 2). Here the marker fragment of 123 bp, indicating the presence of gene *Lr13*, was found in the cultivar – carrier of allele *Ne2^s* Blackhull. This yields a conclusion that the use of a molecular marker *Xgwm630* does not allow identifying allele *Ne2^m*. The impossibility of differentiating between alleles *Ne2^w* and *Ne2^m* while using pairs of primers to microsatellite locus *Xgwm630*

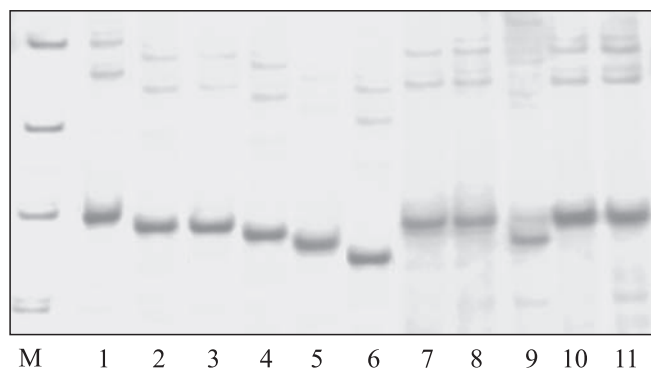


Fig. 1. The PCR results for the identification of the alleles of locus *Xbarc55-2B* in soft wheat cultivars in 9 % PAAG: M – molecular mass marker *pUC19/MspI*; 142 bp: 1 – Chinese Spring (*ne2*); 136 bp: 2 – Vakka (*Ne2^w*), 3 – Sonalika (*Ne2^m*), 7 – Apohey Luhansky, 8 – Antonivka, 9 – Volynska semi-intensive, 10 – Spasivka, 11 – Dar Luhanshchyny; 132 bp: 4 – Dawson (*Ne2^{ms}*); 126 bp: 5 – Blackhull (*Ne2^s*); 122 bp: 6 – Apohey Luhansky

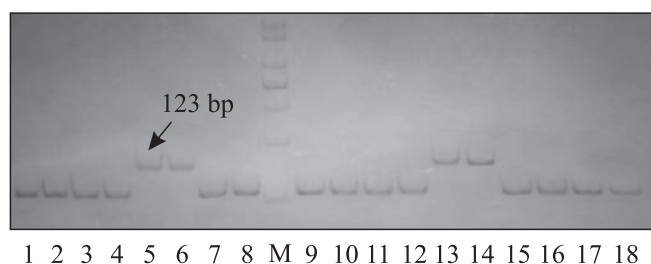


Fig. 2. The PCR results for the identification of gene *Lr13* by marker *Xgwm630-2B* in cultivars – carriers of known alleles of gene *Ne2* and isogenic line of Thatcher cultivar with the resistance gene to leaf rust *Lr13* (*TcLr13*): M – molecular mass marker 50 bp ladder; 1, 2, 9, 10 – Chinese Spring (*ne2*); 3, 11 – Vakka (*Ne2^w*); 4, 12 – Sonalika (*Ne2^m*); 7, 15 – Dawson (*Ne2^{ms}*); 8, 16 – Blackhull (*Ne2^s*); 5, 6, 13, 14 – TcLr13; 17, 18 – Myronivska 808 (*Ne2^{ms}*)

may be explained by rather a great genetic distance between locus *Xgwm630* and gene *Lr13* and gene *Ne2* respectively.

The investigation of 290 soft wheat cultivars allowed identifying five alleles of locus *Xbarc55-2B* of 142, 136, 132, 126 and 122 bp (Table). The revealed alleles of locus *Xbarc55-2B* in cultivars of Ukrainian and Russian selection were referred to the alleles of gene *Ne2* with the corresponding strength in accordance to the data of the PCR-analysis of known cultivars-carriers. The allele of 122 bp, remarkable for Apohey Luhansky cultivar, was not observed among selected known cultivars – carriers of alleles of gene *Ne2*.

Most investigated cultivars (90.7 %) are linear, they are characterized by the presence of one of alleles of

locus *Xbarc55-2B*. A number of cultivars (9.3 %) were found to be non-homogeneous and had two genotypes by alleles of the abovementioned locus. The population cultivars were divided into 4 groups depending on combinations of the genotype. The frequency of cultivars of each group was not high – from 0.3 to 4.5 %. Therefore, all the investigated cultivars may be divided into 9 groups, 4 of which are represented by homogeneous, and 5 – by non-homogeneous cultivars with the combination of two genotypes with different alleles of the abovementioned locus.

The most common allele for soft wheat cultivars of Ukrainian and Russian selection was the allele of locus *Xbarc55-2B* of 132 bp, which corresponded to dominant alleles of genes *Ne2^w* and *Ne2^m*. The frequency of the mentioned allele in the total selection of cultivars was 76.4 % (Fig. 3). As mentioned above, the same group included the wheat cultivars with alleles *Ne2^w* and *Ne2^m*. Rather a high frequency (15.0 %) for cultivars of the studied selection was observed for allele of 132 bp, which corresponds to the dominant allele *Ne2^{ms}*. The least common among cultivars of Ukrainian selection were alleles of 142 bp (1.8 %) and 126 bp (6.7 %), which corresponded to the recessive allele *ne2* and a strong allele of hybrid necrosis *Ne2^s*. The cultivars with genotypes *Ne2^{ms}Ne2^{ms}* and *Ne2^sNe2^s* (Table) should be paid special attention of selection breeders while planning the crossing scheme.

The distribution of alleles of gene *Ne2* in soft wheat cultivars of different regions of Ukraine and RF are of highest interest. There is a significantly higher frequency among cultivars of the south of Ukraine for allele of 136 bp (91.4 %) (*Ne2^{w/m}*) (Fig. 3). In cultivars of Ukrainian selection, it originates from the cultivar Krymka, but the distribution of the mentioned allele is related to the use of such cultivars as Bezostaya1, Ukrainka, Albatros Odesky in the selection process. Allele of 136 bp also prevails in cultivars of the north of Ukraine (50.6 %), the Northern Caucasus (73.3 %), Western Siberia and Povolzhye (68.5 %). Allele of 132 bp (*Ne2^{ms}*) is wide-spread among the cultivars of the north of Ukraine (28.2 %) and the Northern Caucasus (26.7 %). Among the cultivars of the south of Ukraine, allele of 132 bp (*Ne2^{ms}*) is observed with much less frequency ($d = 21.4 \pm 5.3$) compared to the cultivars of the north of Ukraine. The distribution of allele of 132 bp (*Ne2^{ms}*) among cultivars of Ukrainian and Russian selection is related to the use of cultivar Myronivska 808 in the selection process. Allele of 126 bp (*Ne2^s*) is observed for cultivars of the north of Ukraine and Western Siberia,

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The genotypes of soft wheat cultivars of different geographic location by the alleles of locus *Xbarc55-2B*

Size of allele, bp	N	p ± sp, %	Cultivar
142 (<i>ne2</i>)	4	1.4 ± 0.7	Mirlena, Monolog, Milturum 513, Columbia
136 (<i>Ne2w</i> or <i>Ne2m</i>)	211	72.8 ± 2.6	Avroras, Albatros Odesky (un.), Albidum 114, Albidum 12, Antonivka, Artemivka, Astet Luhansky, Bezmezhna, Bezosta 1, Bilotserkivska 198, Biliava, Blahodarka un., Borviy, Bryz, Brihantyna, Bunchuk, Burevisnyk un., Vasylyna, Vatazhok, Vdala, Veselka, Veselopodolianska 499, Vesnianka, Veteran, Vympel un., Vykhovanka un., Vigen, Viktoria un., Volodarka, Harant, Hirka Mistseva (0274), Hoduvalnytsia un., Holubka un., Hospodynina, Hostianum 237, Hostianum 273, Hostianum 274, Hurt, Dalnytska, Dar Luhanshchyny, Darunok, Dovira, Donetska 48, Donsymb, Donska, Driada 1, Diuk, Elehiia, Epokha un., Era, Erytrospermum 127, Erytrospermum 15, Erytrospermum 217, Erytrospermum 2917, Yednist, Zhaivir, Zhuravka, Zadumka, Zamozhnist, Zastava un., Zvytiaga, Zemka, Zemliachka un., Zenitka, Zysk, Zirka, Zlahoda, Zmina, Znakhidka un., Zolotava, Zolotokolosa, Zorepad, Ivanivska awned, Istyna un., Kavkaz, Kazanska 237, Kazanska 285, Kyivska 8, Kyivska awned, Kyianka, Kiriia, Kniahynia Olha, Kolomak 3, Kolomak 5, Krasen, Krasunia un., Krymka Mistseva, Kubanka, Kubanka 2, Kuialnyk, L-326, Lad, Lada un., Lan, Lanovyi, Lebidka, Leleka, Lelia, Lytanivka, Liona, Lira, Luzanivka un., Liubynka, Liutescenc 17, Liutescenc 23397, Liutescenc 7, Melodia, Myronivska Yuvileina, Myronivska 31, Myronivska 40, Milturum 553, Milturum pererod, Misia un., Mudrist, Nasnaha, Nakhodka 4, Nebokrai, Nyva, Nikonia, Obriy, Odeska 3, Odeska 12, Odeska 16, Odeska 26, Odeska 51, Odeska 66, Odeska 117, Odeska 120, Odeska 130, Odeska 132, Odeska 265, Odeska 266, Odeska 267, Odeska 268, Odeska unawned, Odeska red, Odeska semi-dwarf, Odeska awned semi-intensive, Odom, Oksana, Olesia, Olvia, Omska 2, Omska 4, Omska winter, Otaman, Panna, Peresvet, Perlyna lisostepu, Pylypivka, Pysanka, Pivdenna Zoria, Pobeda 50, Povaha, Podiaka, Poliska 90, Polukarlyk 1, Poliovyk, Porada, Poshana, Prybiy, Pryma un., Progress, Prokofievka, Prometei, Rodyna, Rozmai, Rosynka, Svitank 1, Selianka, Symvol un., Syrena un., Skarbnytsia, Skifianka, Skorospelka 1, Skorospelka 3b, Sluzhnytsia un., Snihurka, Snizhana, Sofiyka, Spartanka, Spasivka, Spivanka, Strumok, Sputnytsia, Tradytzia, Turunchuk, Uzhynok, Ukrainka un., Ukrainka poltavaska, Unikum, Fantazia un., Fedorovka, Frehat un., Kharus, Khvylia, Khersonska 99, Khersonska awned, Khersonska unawned, Khyst, Khurtovyna, Tsyhanka, Chaika, Chervona, Chornobryva, Shchedrist, Yuvileina 75, Yunat un., Yakir un., Yatran 60
132 (<i>Ne2ms</i>)	34	11.7 ± 1.9	Bilotserkivska semi-dwarf, Bilosnizhka, Bohdana, Voloshkova, Dobrochyn, Donsky siurpryz, Ekonomka, Ekspromt, Zaporuka, Zustrich, Kalynova, Knopa, Kolos Myronivshchyny, Krasnodarska 99, Lastivka, Lisostepka 75, Liubava un., Myronivska awned, Myronivska 808, Monotyp, Odeska 162, Omska 3, Severna Zoria, Podolianka, Rostavytsia, Tira, Ukrainka, Ukrainka 0246, Ulianivka, Kharkivska 96, Kharkivska 105, Shestopalivka, Yuna, Yasnohirka
126 (<i>Ne2s</i>)	15	5.2 ± 1.6	Bahrationivska, Volynska semi-intensive, Kolektyvna, Kooperatorka, Kryzhynka, Myrych, Mirleben, Myronivska 27, Myronivska 30, Myronivska 33, Myronivska 66, Saratovska 25, Stepova, Favorytka, Yasochka
142 + 136 (<i>ne2</i> + <i>Ne2w/m</i>)	3	1.0 ± 0.6	Dobirna, Smila, Smuhlianka
136 + 132 (<i>Ne2w/m</i> + <i>Ne2ms</i>)	13	4.5 ± 1.2	Banatka, Dykanka, Donetska semi-dwarf, Zagrava un., Kosovytsia, Odeska 133, Myronivska 264, Batko, Sybirska Nyva, Slavna, Stanychna, Ilyichivka, Omska 5

Continued Table

Size of allele, bp	N	p ± sp, %	Cultivar
136 + 126 (<i>Ne2w/m</i> + <i>Ne2s</i>)	4	1.4 ± 0.7	Zoria, Krymka, Nahoroda un., Myronivska 65
136 + 122 (<i>Ne2w/m</i> + ?)	1	0.3 ± 0.3	Apohey Luhansky
132 + 126 (<i>Ne2ms</i> + <i>Ne2s</i>)	5	1.7 ± 0.8	Lybid, Lutescenc 606, Myronivska 61, Myrhard, Lehenda Myronivshchyny
	290		

Note. *Two genotypes with different alleles of locus *Xbarc55-2B* are present in the cultivar.

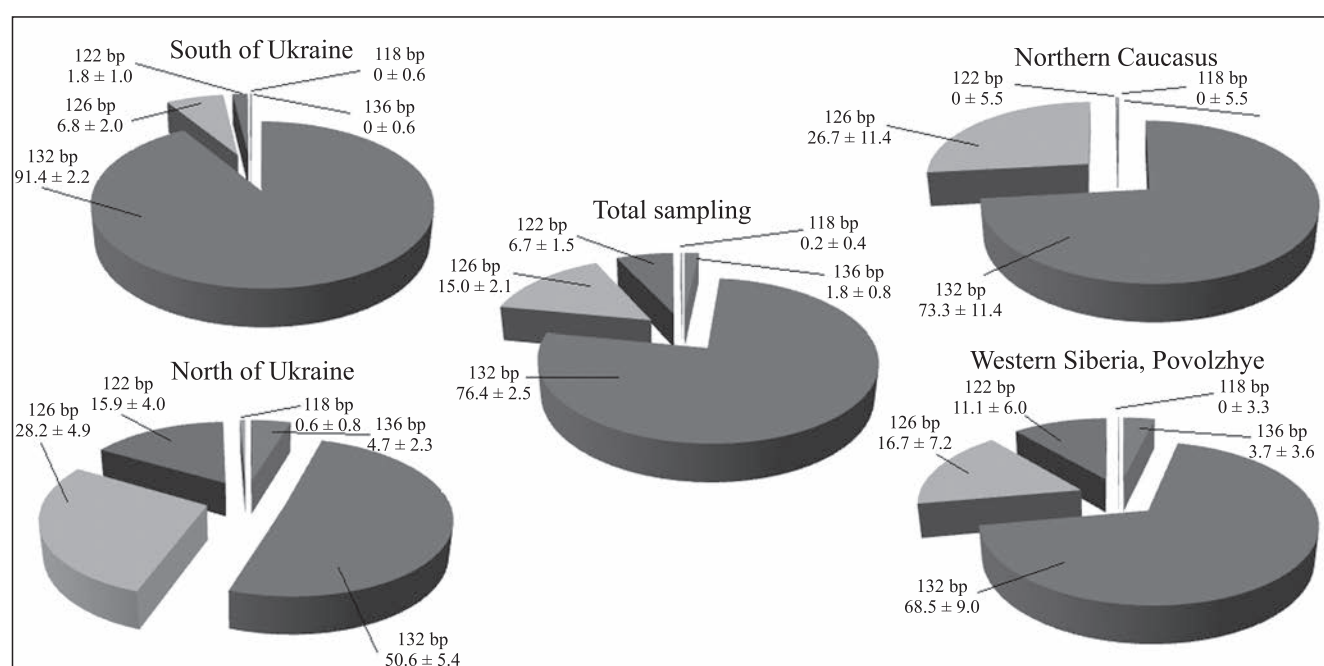


Fig. 3. The frequencies of alleles of locus *Xbarc55-2B* in the total selection of cultivars and selections of cultivars from different regions

Povolzhye much more frequently than for cultivars of the south of Ukraine ($d = 14.1 \pm 4.1$ and $d = 9.3 \pm 6.1$ respectively), the differences are reliable for the former case. The allele of 126 bp was not observed among cultivars of the Northern Caucasus (*Ne2^s*). The source of allele of 126 bp (*Ne2^s*) in wheat cultivars are cultivars Krymka and Kooperatoroka, but the distribution of the mentioned allele among the cultivars of Ukrainian and Russian selection is related to cultivars of the Myronivka Wheat Institute named after V. M. Remeslo, Myronivska Yubileyna and Myronivska 27.

The prevailing distribution of allele of 136 bp (*Ne2^{w/m}*) among the cultivars of the south of Ukraine and the

Northern Caucasus, allele of 132 bp (*Ne2^{ms}*) – among the cultivars of the Northern Ukraine, as well as allele of 126 bp (*Ne2^s*) – among the cultivars of the Northern Ukraine and Western Siberia and Povolzhye may testify to selection and adaptation value of the mentioned alleles of locus *Xbarc55-2B*, more particularly, alleles of genes, bound to the mentioned locus, for conditions of certain regions.

When the matter is related to gene, bound to locus *Xbarc55-2B*, it covers both the gene of hybrid necrosis *Ne2* and other genes of chromosome 2BS, which may have relevant adaptive or selective significance, for instance, genes of sensitivity to photoperiod (*Ppd-*

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B1), resistance to premature germination of seeds in the spike (*Qphs-2B*) and resistance to fungal diseases (*Sr19*, *Sr36*, *Sr40*, *Yr27*, *Lr13*, *Lr23*, *Pm26*, *Pm42*, *Pm49*, *Tsc2*) [16].

CONCLUSIONS

The alleles of locus *Xbarc55-2B*, bound to the gene of hybrid necrosis *Ne2*, were used to identify genotypes of 290 cultivars of soft wheat of different geographic origin. The correspondence of different alleles of locus *Xbarc55-2B* to alleles of gene *Ne2*, different in their strength, was defined: 142 bp – *ne2*, 136 bp – *Ne2^{w/m}*, 132 bp – *Ne2^{ms}*, 126 bp – *Ne2^s*. The distribution of the identified alleles of gene *Ne2* among the wheat cultivars of Ukrainian and Russian selection was demonstrated.

Ідентифікація та поширення алелів гена гібридного некрозу *Ne2* у сортів м'якої пшениці (*Triticum aestivum* L.)

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Мета. Визначити відповідність різних алелів локусу *Xbarc55-2B* неоднаковим за силою алелям гена *Ne2* та дослідити розподіл алелів локусу *Xbarc55-2B* і відповідних алелів гена *Ne2* з-поміж сортів пшениць української і російської селекції.

Методи. Полімеразна ланцюгова реакція, електрофорез у ПААГ, статистичні методи. **Результати.** За алелями локусу *Xbarc55-2B*, зчепленого з геном гібридного некрозу *Ne2*, ідентифіковано генотипи 290 сортів пшениці м'якої різного географічного походження. Визначено відповідності різних алелів локусу *Xbarc55-2B* певним за силою алелям гена *Ne2*: 142 п. н. – *ne2*, 136 п. н. – *Ne2^{w/m}*, 132 п. н. – *Ne2^{ms}*, 126 п. н. – *Ne2^s*. Показано розподіл ідентифікованих алелів гена *Ne2* серед сортів пшениць української і російської селекції. **Висновки.** Встановлено перевагу певних алелів для сортів різних регіонів, що може свідчити про їхню селекційну і адаптивну цінність.

Ключові слова: *Triticum aestivum* L., ген гібридного некрозу *Ne2*, мікросателітний аналіз.

Идентификация и распространение аллелей гена гибридного некроза *Ne2* у сортов мягкой пшеницы (*Triticum aestivum* L.)

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Цель. Определить соответствие различных аллелей локуса *Xbarc55-2B* неодинаковым по силе алелям гена *Ne2* и исследовать распределение аллелей локуса *Xbarc55-2B* и соответствующих аллелей гена *Ne2* среди сортов пшеницы украинской и российской селекции. **Методы.** Полимеразная цепная реакция, электрофорез в ПААГеле, статистические методы. **Результаты.** По алелям локуса *Xbarc55-2B*, сцеп-

ленного с геном гибридного некроза *Ne2*, идентифицированы генотипы 290 сортов пшеницы мягкой различного географического происхождения. Выявлено соответствие аллелей локуса *Xbarc55-2B* определенным по силе алелям гена *Ne2*: 142 п. н. – *ne2*, 136 п. н. – *Ne2^{w/m}*, 132 п. н. – *Ne2^{ms}*, 126 п. н. – *Ne2^s*. Показано распределение идентифицированных аллелей гена *Ne2* среди сортов пшеницы украинской и российской селекции. **Выводы.** Отмечено преимущество определенных аллелей для сортов различных регионов, что может свидетельствовать об их селекционной и адаптивной ценности.

Ключевые слова: *Triticum aestivum* L., ген гибридного некроза *Ne2*, микросателлитный анализ.

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