INTRODUCTION

Hybrid necrosis of wheat is a premature gradual perishing of leaves or plants of wheat F1 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 ne1ne1Ne2sNe2s and Ne1sNe1sne2ne2 [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 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 Xbarc74-5 is bound to Ne1 at a genetic distance of 2.0 cM, and Xbarc55-2B – 3.2 cM from Ne2.

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 – Ne2w/m, 132 bp – Ne2ms, 126 bp – Ne2s. 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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The reaction conditions: denaturation at 95 ºC, mineral oil were applied above the reaction mixture.

(72 ºC and 30 s), elongation (3 min). The amplification products (10 µl aliquots of PCR-mixture) were fractioned in 12 % non-denaturating polyacrylamide gel (PAAG) in buffer 1 × 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 video-system 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 (Ne2w), Sonalika (Ne2s), Dawson (Ne2m) and Blackhull (Ne2). 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 Ne2w and Ne2m correspond to allele of 136 bp, allele of gene Ne2w – 132 bp and allele of gene Ne2s to allele of 126 bp. (Fig. 1). It was not possible to differentiate cultivars – carriers of alleles Ne2w and Ne2m for the pair of primers by locus Xbarc55-2. Allele of 136 bp of locus Xbarc55-2B was marked as Ne2w.

There are literature data on the fact that the resistance gene Lr13 of adult plants to leaf rust is bound to allele Ne2w [13, 14]. The differentiation of alleles Ne2w and Ne2m 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 (Ne2s) (Fig. 2). Here the marker fragment of 123 bp, indicating the presence of gene Lr13, was found in the cultivar – carrier of allele Ne2 Blackhull. This yields a conclusion that the use of a molecular marker Xgwm630 does not allow identifying allele Ne2w. The impossibility of differentiating between alleles Ne2w and Ne2m while using pairs of primers to microsatellite locus Xgwm630...
The investigation of 290 soft wheat cultivars allowed identifying five alleles of locus \textit{Xbarc55-2B} of 142, 136, 132, 126 and 122 bp (Table). The revealed alleles of locus \textit{Xbarc55-2B} in cultivars of Ukrainian and Russian selection were referred to the alleles of gene \textit{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 \textit{Ne2}.

Most investigated cultivars (90.7 \%) are linear, they are characterized by the presence of one of alleles of locus \textit{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 \textit{Xbarc55-2B} of 132 bp, which corresponded to dominant alleles of genes \textit{Ne2} and \textit{Ne2m}. 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 \textit{Ne2m} and \textit{Ne2m}. 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 \textit{Ne2m}. 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 \textit{ne2} and a strong allele of hybrid necrosis \textit{Ne2}. The cultivars with genotypes \textit{Ne2mNe2m} and \textit{Ne2Ne2} (Table) should be paid special attention of selection breeders while planning the crossing scheme.

The distribution of alleles of gene \textit{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 \%) (\textit{Ne2m}) (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 Bezostaya 1, 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 Povolzhie (68.5 \%). Allele of 132 bp (\textit{Ne2m}) is widespread 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 (\textit{Ne2m}) 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 (\textit{Ne2m}) among cultivars of Ukrainian and Russian selection is related to the use of cultivar Myronivska 808 in the selection process. Allele of 126 bp (\textit{Ne2}) is observed for cultivars of the north of Ukraine and Western Siberia,
The genotypes of soft wheat cultivars of different geographic location by the alleles of locus \textit{Xbarc55-2B}

<table>
<thead>
<tr>
<th>Size of allele, bp</th>
<th>N</th>
<th>p ± sp, %</th>
<th>Cultivar</th>
</tr>
</thead>
<tbody>
<tr>
<td>142 (\textit{ne2})</td>
<td>4</td>
<td>1.4 ± 0.7</td>
<td>Mirlena, Monolog, Milturum 513, Columbia</td>
</tr>
<tr>
<td>132 (\textit{Ne2ms})</td>
<td>34</td>
<td>11.7 ± 1.9</td>
<td>Biloserkivska semi-dwarf, Bilosnizhka, Bohdana, Voloshkova, Dobrochyn, Donskyi siurpryz, Ekonomka, Eksrompt, Zaporuka, Zustrich, Kalynova, Knopa, Kolos Myrnyvivschnhy, Krasnodarska 99, Lastivka, Lisostepka 75, Liubava un., Myrnyvivska awned, Myrnyvivska 808, Monotyp, Odeska 162, Omska 3, Severna Zoria, Podolianka, Rostavytsia, Tira, Ukrainka, Ukrainka 0246, Ulianka, Kharkivska 96, Kharkivska 105, Shhestopalivka, Yuna, Yasnolhira</td>
</tr>
<tr>
<td>126 (\textit{Ne2s})</td>
<td>15</td>
<td>5.2 ± 1.6</td>
<td>Bahrinationsvska, Volynska semi-intensive, Kolektyvna, Kooperatorka, Kryzhynka, Myrych, Mirleben, Myrnyvivska 27, Myrnyvivska 30, Myrnyvivska 33, Myrnyvivska 66, Saratsvysia 25, Stepova, Favorytka, Yasoshcha</td>
</tr>
<tr>
<td>142 + 136 (\textit{ne2} + \textit{Ne2w/m})</td>
<td>3</td>
<td>1.0 ± 0.6</td>
<td>Dobirna, Smila, Smuhlanka</td>
</tr>
<tr>
<td>136 + 132 (\textit{Ne2w/m} + \textit{Ne2ms})</td>
<td>13</td>
<td>4.5 ± 1.2</td>
<td>Banatka, Dykanka, Donetska semi-dwarf, Zagrava un., Kosovytsia, Odeska 133, Myrnyvivska 264, Batko, Sybirskia Nyva, Slavna, Stanychna, Ilyichivka, Omska 5</td>
</tr>
</tbody>
</table>
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 ($Ne2s$). The source of allele of 126 bp ($Ne2s$) in wheat cultivars are cultivars Krymka and Kooperatorka, 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 ($Ne2w/m + Ne2s$) among the cultivars of the south of Ukraine and the Northern Caucasus, allele of 132 bp ($Ne2ms + Ne2s$) – among the cultivars of the Northern Ukraine, as well as allele of 126 bp ($Ne2s$) – 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 $Xbarc55-2\circ$, 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 $Xbarc55-2\circ$, 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 ($Ppd$-
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 – Ne2w, 132 bp – Ne2m, 126 bp – Ne2. The distribution of the identified alleles of gene Ne2 among the wheat cultivars of Ukrainian and Russian selection was demonstrated.

REFERENCES