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GENOTYPING OF UKRAINIAN COMMON WHEAT CULTIVARS USING THE MARKER OF THE *Lr48* GENE CONFERRING MODERATE RESISTANCE TO LEAF RUST

Aim. Common wheat (*Triticum aestivum* L.) is one of the most important and widely cultivated crops over the world. For a lot of wheat diseases introduction of resistance genes is considered to be the most rational way to diminish yield losses and control spread of causal agents. The aim of this research was to study a sample of Ukrainian common wheat cultivars with the use of the molecular genetic marker for the *Lr48* gene. **Methods.** DNA samples of 46 common wheat cultivars developed in the Remeslo Myronivka Institute of Wheat (RMIW) of National Academy of Agrarian Sciences jointly with the Institute of Plant Physiology and Genetics of National Academy of Sciences of Ukraine were analyzed with the use of the marker IWB70147. **Results.** It was revealed that 15 out of 46 (or 32.6 %) cultivars carried resistance-associated allele of the marker. **Conclusions.** It was revealed that the resistance-associated allele of the marker of the *Lr48* gene is present in Ukrainian common wheat cultivars developed in the Forrest Steppe zone of Ukraine. The possible source of the resistance allele is ‘Mironovskaya 808’ which is in the pedigree of many Ukrainian and world wheat cultivars. The data obtained in this research can be used in breeding programs to select sources of moderate adult plant resistance. Cultivars ‘Yuviliar Myronivskii’, ‘Volodarka’ and ‘Pamyati Remesla’ with adult leaf rust resistance conferred by the *Lr48* gene also carry resistance associated allele of the *Lr34/Yr18/Pm38/Sr57/Bdv1* gene.

Keywords: molecular markers, wheat, resistance genes, adult plant resistance.

Common wheat (*Triticum aestivum* L.) is one of the most important and widely cultivated crops over the world [1]. For a lot of wheat diseases introduction of resistance genes is considered to be the most rational way to diminish yield losses and control spread of causal agents [2, 3]. So genetic basic and new sources of resistance are of concern to scientists and breeders [4–6]. One of the most widespread fungal diseases of wheat is leaf or brown

rust (the causal agent is *Puccinia triticina* Erikss.) [5–7]. In a number of studies, the genetic background of race specific resistance against leaf rust was revealed [8–10]. Although this type of resistance can be easily detected in the field and significantly reduces the disease symptoms, it is not durable for a prolonged period of time: for the majority of known genes conferring race specific resistance virulent races eventually emerged [6, 11]. Adult leaf rust resistance genes are on the other hand far less conspicuous in field and confer only a moderate level of resistance, however races of *P. triticina* virulent to most of known adult plant resistance genes have not been discovered yet [6]. Both kinds of leaf rust resistance genes are used in modern breeding strategies like “pyramiding” and “marker-assisted selection” [12, 13].

Molecular genetic markers of adult plant resistance gene *Lr34/Yr18/Pm38/Sr57/Bdv1* [14], tan spot (in)sensitivity genes *Tsn1* and *Tsc2* [15] and *Fusarium* head blight resistance gene *TDF_076_2D* [16] were used for genotyping of Ukrainian common wheat cultivars. As a result of the statistical processing of the obtained data the correlation suggesting a major quantitative trait locus on chromosome 2BS conferring durable field leaf rust resistance was revealed [17]. One of the possible genes located on chromosome 2BS and being able to confer such a resistance is gene *Lr48* [17].

Gene *Lr48* was first described as a race specific resistance gene [18]. Further research, however, revealed the nature of resistance conferred by the gene as moderate and adult [19]. The gene was mapped with use of SSR markers, although in different publications it is localized either on the long arm of chromosome 2B [18, 19] or on the short one [20]. The latest and the most durable set of markers was developed to use KASP technique; in this research the gene was once again localized on chromosome 2BS; among the markers used *IWB70147* was considered to be the most accurate [17].

The *aim* of this research was to study a sample of Ukrainian common wheat cultivars with the use

of the molecular genetic marker for the *Lr48* gene.

Materials and methods

DNA extracted from seeds of 46 wheat cultivars developed in the Remeslo Myronivka Institute of Wheat (RMIW) of National Academy of Agrarian Sciences jointly with the Institute of Plant Physiology and Genetics of National Academy of Sciences of Ukraine were analyzed. The complete list of the cultivars is shown in table. For DNA extraction the samples of 25–30 mg obtained from grinding 5–7 seeds were used. Further procedure included the use of the Diatom TM DNA Prep100 DNA isolation kit (the sales representative in Ukraine is Neogene® Company) according to the standard protocol. PCR was performed using GenPak® PCR Core Kits (the sales representative in Ukraine is Neogene® Company) according to the manufacturer's recommendations. The primers flanking the marker *IWB70147* used in this study were the following: *IWB70147_F2* (5'cgcctacacccttatgtacc3') and *IWB70147_R* (5'taccaatctagagtaacattaccgcaaa3') [18]. PCR was performed according to the following protocol: previous denaturation and Hot Start DNA-polymerase activation at 94°C for 6 min.; 32

cycles including denaturation at 94°C for 50 s, annealing at 63°C for 25 s and elongation at 72°C for 30 s; final elongation at 72°C for 5 min. The amplified fragments in case of the resistance-associated allele of the marker were ~62 bp in length (further marked as '+', see Fig. and table) and no amplified fragments were expected to obtain otherwise (further marked as '-', see Fig. and table) [17].

PCR results were visualized by electrophoresis in 2–2.5 % agarose gel in 0.5 × TBE buffer with subsequent staining with ethidium bromide and use of the gel-visualization system VISION Gel (Fig.).

Results and discussion

The marker used in this research was originally developed for the KASP technique [17]. Complementary to the resistance-associated allele primer *IWB70147_F1* with common primer *IWB70147_R* were successfully used as a marker for conventional PCR (fig.). The primer *IWB70147_F1* complementary to the allele '-' in combination with the *IWB70147_R* primer did not reveal any polymorphism among the cultivars tested (data not shown). So in this research *IWB70147* was used as a dominant conventional PCR marker (fig.).

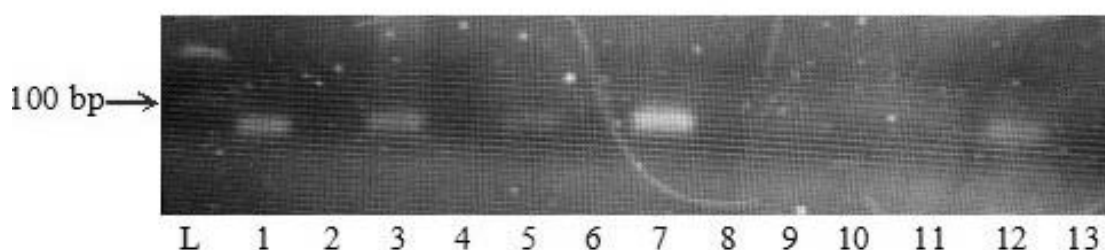


Fig. Example of PCR products with the primers flanking the marker *IWB70147*. The samples used in PCR are following: 1 – 'Myronovskaya 808'; 2 – 'Thatcher'; 3 – 'Dobirna'; 4 – 'Oberig Myronivskii'; 5 – 'Lasunia'; 6 – 'Myrlena'; 7 – 'Khazarka'; 8 – 'Kolumbiya'; 9 – 'Bohdana'; 10 – 'Snigurka'; 11 – 'Ukrainka'; 12 – 'Yasnogirka'; 13 – 'Madyarka'; L – 100 bp ladder.

According to the data on the allelic state of the marker *IWB70147* for the cultivars studied, the resistance conferred by the *Lr48* gene is quite common at least for the wheat varieties developed in the Forrest Steppe zone of Ukraine (table).

It was revealed that 15 out of 46 (or 32.6 %) cultivars tested carried the '+' allele of the marker (table). Among the cultivars with the resistance allele of the gene is 'Mironovskaya 808' known to be in the pedigree of many Ukrainian and European common wheat cultivars [21]. The cultivars 'Yuvliar Myronivskii', 'Volodarka' and 'Pamyati Remesla' with the allele '+' of the marker *IWB70147*

have been found to carry resistance allele of the *Lr34/Yr18/Pm38/Sr57/Bdv1* gene as well [14], making them useful in breeding aimed at "pyramiding" of leaf rust resistance factors [12]. Besides that, 'Volodarka' carries alleles of the *Tsn1* and *Tsc2* genes associated with insensitivity to toxins *Pyrenophora tritici-repentis* (Died.) Drechsler, (1923) [15]. In addition, 'Yasnogirka', 'Myronivska 28', 'Svitanok Myronivskii' and 'Zolotokolosa' besides the '+' allele of the marker *IWB70147* also carry alleles of the *Tsn1* and *Tsc2* genes associated with insensitivity to toxins *P. tritici-repentis* [15].

Table. Allelic state of the marker *IWB70147* for gene *Lr48* in a sample of Ukrainian common wheat cultivars

Cultivar name	Allelic state of the marker <i>IWB70147</i>	Cultivar name	Allelic state of the marker <i>IWB70147</i>	Cultivar name	Allelic state of the marker <i>IWB70147</i>
Bohdana	-	Myronivska 264	+	Pereyaslavka	-
Demetra	-	Myronivska 27	-	Podolianka	-
Dobirna	+	Myronivska 28	+	Pyvna	-
Dostatok	+	Myronivska 29	-	Smuglianka	-
Eksprompt	-	Myronivska 31	-	Snigurka	-
Favorytka	-	Myronivska 33	-	Snizhana	-
Khazarka	+	Myronivska 61	-	Sviatkova	-
Khurtovyna	-	Myronivska 65	-	Svitanok Myronivskii	+
Kolos Myronivshchyny	-	Myronivska 66	+	Ukrainka	-
Kolumbiya	-	Myronivska 67	-	Vesnianka	+
Kryzhynka	-	Myronivska rannios-tygla	-	Vesta	-
Lasunia	+	Myronivska Storichna	-	Volodarka	+
Legena Myronivshchyny	-	Myronovskaya 808	+	Voloshkova	-
Madyarka	-	Oberig Myronivskii	-	Yasnogirka	+
Myrlena	-	Pamyati Remesla	+	Yuvliar Myronivskii	+
				Zolokolosa	+

Conclusions

It was revealed that the resistance-associated allele of the marker of the *Lr48* gene is present in Ukrainian common wheat cultivars developed in the Forrest Steppe zone of Ukraine. The possible source of the resistance allele is ‘Mironovskaya 808’ which is in the pedigree of many Ukrainian and world

wheat cultivars. The data obtained in this research can be used in breeding programs to select sources of moderate adult plant resistance. Cultivars ‘Yuvliar Myronivskii’, ‘Volodarka’ and ‘Pamyati Remesla’ with adult leaf rust resistance conferred by the *Lr48* gene also carry resistance associated allele of the *Lr34/Yr18/Pm38/Sr57/Bdv1* gene.

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ГЕНОТИПУВАННЯ СОРТІВ ПШЕНИЦІ М'ЯКОЇ УКРАЇНСЬКОЇ СЕЛЕКЦІЇ ЗА ДОПОМОГОЮ МАРКЕРА ГЕНА *LR48*, ЩО ЗАБЕЗПЕЧУЄ ПОМІРНУ СТІЙКІСТЬ ДО БУРОЇ ІРЖІ

Мета. Пшениця м'яка (*Triticum aestivum* L.) є однією з найбільш важливих і широко вирощуваних сільськогосподарських культур у світі. Для багатьох хвороб пшениці впровадження генів стійкості вважають найраціональнішим шляхом зменшити втрати урожаю і контролювати поширення збудників. Метою цього дослідження було вивчення вибірки сортів пшениці м'якої української селекції за допомогою маркера гена *Lr48*. **Методи.** Зразки ДНК 46 сортів пшениці м'якої створених у Миронівському інституті пшениці ім. Ремесла Національної академії аграрних наук України разом із Інститутом фізіології рослин і генетики Національної академії наук України були досліджені за допомогою маркера ІWB70147. **Результати.** Було виявлено, що 15 з 46 (або 32,6 %) сортів несуть асоційований зі стійкістю алель маркера. **Висновки.** Було виявлено, що асоційований зі стійкістю алель маркера гена *Lr48* присутній у сортів пшениці м'якої, створених у лісостеповій зоні України. Можливим джерелом алеля стійкості є Миронівська 808, яка зустрічається у родовах багатьох українських і світових сортів. Дані, отримані в цьому дослідженні, можуть бути використані в селекційних програмах для вибору джерел помірної дорослої стійкості. Сорти Ювіляр Миронівський, Володарка і Пам'яті Ремесла із дорослою стійкістю, яку забезпечує ген *Lr48* також несуть пов'язаний зі стійкістю алель гена *Lr34/Yr18/Pm38/Sr57/Bdv1*.

Ключові слова: молекулярні маркери, пшениці, гени стійкості, доросла стійкість рослин.