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Analysis of Ukrainian Polissya and Forest-steppe winter wheat (*Triticum aestivum* L.) cultivars for the presence of "resistant" allelic state of non-race-specific disease resistance locus *Lr34/Yr18/Pm38*

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ABSTRACT

Resistance against rusts and powdery mildew is one of the most important traits of modern wheat cultivars, because in some years these diseases can significantly reduce crop yields. The genes that confer durable non-race-specific resistance are of interest to many breeding programs. One of them is *Lr34/Yr18/Pm38*. This multiple resistance locus is associated with partial and durable resistance to rust diseases, powdery mildew and tolerance to barley yellow dwarf virus. We used marker *caISBP1* (insertion site-based polymorphism marker) specific for the resistance, associated with the *Lr34/Yr18/Pm38* gene. It is a co-dominant marker between loci coding ABC-transporter and cytochrome *P450* in the region involved in resistance expression. We investigated a collection of 28 winter wheat cultivars of NSC ''Institute of Agriculture NAAS Ukraine'' developed in different periods. The "resistant" allelic state of the marker *caISBP1* was identified in 11 cultivars (39% of the total number). The "susceptible" allelic state (the Lr34- allele) was identified in 9 wheat cultivars (32%) and 8 (29%) cultivars were heterozygous. Our investigation confirmed the presence of the Lr34+ allele in Polissya and Forest-Steppe winter wheat cultivars.

***Keywords*:** rust diseases, adult plant resistance, winter wheat

Introduction

A search for cultivars can be donors of resistance genes is a time-consuming but important task of wheat breeding. For effective selection, a breeder should have more or less solid data about the resistance genes and the opportunity for their quick identification during the breeding process. Determining of resistance associated with some genes by the phenotype may be inexact and time-consuming. Detection of resistance gene accelerates with the use of molecular DNA markers which can rapidly identify allelic state of a gene in laboratory with high precision. Using DNA markers in research of resistance gene reduces the time and increases efficiency and accuracy of the investigation in the most cases. The rust diseases of wheat are caused by fungi of the order *Uredinales*. There are three species of wheat rusts: leaf rust (*Puccinia triticina* f. sp. *tritici*), stem rust (*P.graminis* f. sp. *tritici*) and yellow rust (*P. striiformis* f. sp. *tritici*). In some years yield losses due leaf rust could reach 15-20%, stem rust – 60-70%, yellow rust – 20% and more (Peresypkin et al. 1990; Bublyk et al. 1999).

Rust fungi suppress the process of synthesis of HMW glutenin components in kernels which affects bread-making properties. Also they decline the rate of synthesis and deposition of starch and proteins in endosperm causing puny grain (Kolyuchyy et al. 2007).

Breeders can use wide non-race-specific adult plant resistance (APR) genes which stay effective for a long period. APR is associated with reduction of disease development, slowdown of pathogen haustorium penetration in cells of host plant (Krattinger et al. 2009).

Lr34 is one of the most important APRs genes in wheat breeding due to its wide spectrum resistance and stability. It is present in about 50% of cultivars worldwide and was a component of resistance of highly productive cultivars at the start of green revolution (Hoisington et al. 1999). The *Lr34* gene has been staying effective against rusts more than 100 years (Krattinger et al. 2009; Dakouri et al. 2010).

This multiple resistance locus is associated with partial and durable resistance to rust diseases (Dyck et al. 1987; McIntosh et al. 1992; Singh 1992; Kolmer et al. 2011), powdery mildew (*Blumeria graminis* (DC.) Speer) and tolerance to barley yellow dwarf virus (Spielmeyer et al. 2005; Singh 1993). The gene was localized at the short arm of the *7D* chromosome of wheat (Dyck et al. 1987). The *Lr34* locus putatively codes the ATP-binding cassette (ABC)-transporter, a protein localized at the cytoplasmic membrane (Krattinger et al. 2009). A number of molecular marker linked to this gene have been developed (Bossolini et al. 2006, Lagudah et al. 2009). Among them there are the markers localized in the *Lr34* locus or the regulated ones (Dakouri et al. 2010). We used the marker *caISBP1* (insertion site-based polymorphism marker) specific for the resistance, associated with the *Lr34/Yr18/Pm38* gene. It is a co-dominant marker between the ABC-transporter and cytochrome *P450* in region involved in resistance expression (Dakouri et al. 2010).

The aim of our investigation was detecting the allelic state of the *Lr34/Yr18/Pm38* locus using DNA markers because cultivars of NSC "Institute of Agriculture NAAS" have never been investigated for the presence of this resistance. The resistance-associated allele of the *Lr34/Yr18/Pm38* gene can be involved in breeding process to obtain cultivars with resistance to multiple pathogens in Polissya and Forest-Steppe due the positive influence of its climatic conditions on *Lr34* resistance expression.

Materials and methods

The DNA was extracted from kernels of 28 winter wheat cultivars developed in periods from 1971 to 1990 and 1992 – 2012 in Cereal Breeding and Seed Growing Department of the National Science Centre "Institute of Agriculture NAAS Ukraine": Polesskaya-90, Polesskaya-95, Kolectivnaya-77, Shchedraya-Polesya, Polesskaya-71, Polesskaya-70, Polesskaya-80, Polesskaya-87, Polesskaya-107, Polesskaya-bezostaya, Kievskaya-73, Polesskaya-29, Polesskaya-1259, Polesskaya-92, Miryutinka, Zhuravka, Stolichna, Epilog, Gnom, Krayevyd, Artemida, Kopilivchanka, Analog, Benefis. The cultivars Olgana, IZ49-12 (Kesaria-Poliska) and IZ15-12 (Pamyati-Girka) are under field trials. The cultivar Kievskaya-polukarlikovaya was developed in cooperation with the Institute of Plant Physiology and Genetics of NAS (Kiev, Ukraine). Seeds were obtained from the collection of NSC "Institute of Agriculture NAAS" and the National Centre of Plant Genetic Resources (Kharkov, Ukraine). These cultivars were released for growing condition of Forest-Steppe and Polissya agroclimatic zones.

DNA was extracted using Diatom™ DNA Prep 100 (NEOGENE®, Ukraine) kit by the standard protocol from the bulk of 7 kernels. We used allele-specific marker *caISBP1* (insertion site based polymorphism marker) for the identification of the allelic state of the *Lr34/Yr18/Pm38* locus (Dakouri et al. 2010). This codominant marker is located between the *Lr34* locus which encodes the ABC-transporter and first cytochrome *P450* in the site effecting the resistance expression. The GenPak® PCR Core (NEOGENE®, Ukraine) was used for PCR. The results were visualized by electrophoresis in 2-2,5% agarose gel with 1xTBE buffer and stained with ethidium bromide. The amplified DNA fragments of 509 b.p. in length were obtained in case of "resistance" allelic state of the marker *caISBP1* (the *Lr34*+ allele). In case of the "susceptible" allelic state of the marker (the *Lr34*- allele) fragments of 391 b.p. in length were observed (Dakouri et al. 2010). The cultivars Chinese Spring (the *Lr34*+ allele, *St*+) and Renan (the *Lr34*- allele, *St*-) were used as standards. Marker of molecular mass was GeneRulerTM 50 bp DNA Ladder ready-to-use (Fermentas, Lithuania).

Results and discussion

Among 28 cultivars developed in the Institute of Agriculture 11 showed the *Lr34*+ allele of the marker *caISBP1*. They are Stolichna, Artemida, Analog, Benefis, Polesskaya- 70, Polesskaya-bezostaya, Kievskaya-73, Kievskaya - polukarlikovaya, Polesskaya - 80, Zhuravka, IZ15-12, (Table 1). On the Figure 1 represented the electropforegrams of several cultivars showed different results.

In 9 cultivars we detected the "susceptible" allelic state of the marker *caISBP1*: Polesskaya-90, Kolectivnaya-77, Shchedraya-Polesya, Epilog, Gnom, Krayevyd, Kopilivchanka, Olgana and Polesskaya-1259. Heterozygosity was detected in 8 samples: Polesskaya-95, Polesskaya-71, Polesskaya-87, Polesskaya-107, Polesskaya-29, Polesskaya-92, Miryutinka, IZ49-12. Resistance associated with *Lr34* is common in cultivars developed in period before 1990 and 1992-2012 equally.

Our results are in agreement with the previous publication of Karelov et al. (2011) showing that the

Lr34+ allele was widely spread among Ukrainian bread wheat cultivars. A sufficiently high resistance conferred by *Lr34* in cultivars adapted for the Polissya and Forest-Steppe gives evidence of its broad adaptive value, which does not lose the value nowadays. The specificity of breeding process in the Institute of Agriculture (using moderate infectious backgrounds) may account for selection of breeding material with the *Lr34*+ allele. It is known, that main genetic source of *Lr34* resistance was cultivar Bezostaya1, used as a parental component by breeders. This cultivar has a unique combination of adaptability, disease resistance and high bread making quality (Morgounov et al. 2011). Analysis of seed storage proteins loci of investigated cultivars showed the presence of gliadin and HMW glutenin alleles that possibly derived from Bezostaya1, which could be selected due to high bread-making quality (Zaika et al. 2012, Labuschagne et al. 2002).

Thus we performed the investigation of 28 winter wheat cultivars of NSC ‘’Institute of Agriculture NAAS Ukraine” developed in different periods. For molecular diagnostic we used the loci-specific marker *caSNBP1* of the *Lr34/Yr18/Pm38* gene conferring APR against several phytopathogens. Presence of the resistance-associated allele of the marker was identified in 11 cultivars (39% of the total number). These cultivars can be used in breeding programs as donors of the resistance conferred by the *Lr34/Yr18/Pm38* gene.

Table 1 Allelic state of *Lr34/Yr18/Pm38* loci in winter bread wheat cultivars of NSC “Institute of Agriculture NAAS”.

Cultivar	Year of release	Allelic state	Cultivar	Year of release	Allelic state
Polesskaya-71	1971	+/-	Polesskaya-95	1996	+/-
Polesskaya-70	1974	+	Kopilivchanka	2003	-
Kievskaya-73	1974	+	Stolichna	2005	+
Kolectivnaya-77	1974	-	Gnom	2007	-
Kievskaya-Polukarlikovaya	1977	+	Artemida	2008	+
Miryutinka	1978	+/-	Analog	2008	+
Polesskaya-Bezostaya	1981	+	Benefis	2008	+
Polesskaya-80	1982	+	Epilog	2009	-
Shchedraya-Polesya	1986	-	Krayevyd	2012	-
Polesskaya-87	1990	+/-	Olgana		-
Polesskaya-92	1992	+/-	Zhuravka		+
Polesskaya-90	1994	-	Polesskaya-107		+/-
Polesskaya-1259	1995	-	IZ15-12		+
Polesskaya-29	1996	+/-	IZ49-12		+/-

«+» – “resistant” allelic state, «-» – “susceptible” allelic state, «+/-» – heterozygous for the Lr34+ allele) with the marker *caISBP1*

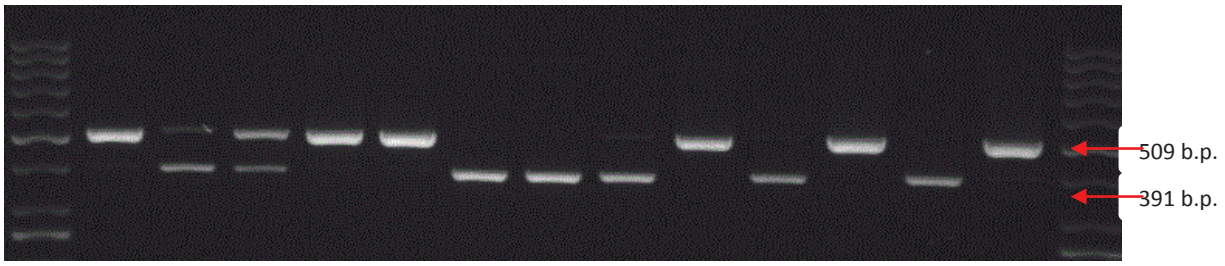

Figure 1 Electrophoregram of PCR products, obtained with DNA samples of bread winter wheat and primers that flank marker *caISBP1*: *1*– Polesskaya-80; *2* – Polesskaya-87; *3* – Polesskaya-107; *4* – Zhuravka; *5* – Stolichna; *6* – Epilog; *7* – Gnom; *8* – Krayevyd; *9* – Artemida; *10* – Kopilivchanka; *11* – Analog; *12* – Renan (St-); *13* – Chinese Spring (St+); L – marker of molecular weight (50 bp DNA Ladder)

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