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Physiologic specialization of *Puccinia triticina* Erikss. and effectiveness of *Lr*-genes in the south of Ukraine during 2013-2014

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Leaf rust is the most widespread and frequently occurring fungal disease of wheat (*Triticum aestivum* L.) in Ukraine and worldwide. The information about the effectiveness of *Lr*-genes and also the consequent monitoring of virulence dynamics is necessary for the successful wheat breeding for leaf rust resistance. In 2013-2014 pathotype composition and virulence analysis was studied both on the standard differential set and on the North American System of Nomenclature. According to the standard differential set, 12 phenotypes were identified, of which the most common were 77 (75%) and 144 (6%). A total of 40 phenotypes were identified on the North American Nomenclature. Phenotypes TGTT (24%) and TJTT (8%) were the most frequent, TRTT (1.5%) and TSTT (1.5%) were within the broadest spectrum of virulence among the isolates found in the south of Ukraine. For virulence analysis we used wheat lines of 'Thatcher' that are near-isogenic for 24 leaf rust resistance genes and additionally four cultivars/lines. No virulence to *Lr19* was found, whereas increasing virulence to *Lr9* was detected (13%). Low frequency of virulence was observed to *Lr29* (11%) and *Lr47* (21%), high level of virulence was detected to other genes. The effectiveness of 53 known *Lr*-genes was studied at the seedling and the adult plant stages. Most of them were not effective against leaf rust. Genes *Lr9*, *Lr19*, *Lr29*, and *Lr47* were highly effective both at the seedling stage and at adult plant stage. Genes *Lr24*, *Lr42*, *Lr50*, *Lr51*, and *Lr56* were effective only at the adult plant stage.

Key words: Leaf rust, resistance, *Triticum aestivum*, virulence analysis, wheat.

INTRODUCTION

Leaf rust (caused by *Puccinia triticina* Erikss.) is the most widespread and frequently occurring fungal disease of wheat (*Triticum aestivum* L.) in Ukraine and throughout the world (Babayants, 2011; Huerta-Espino et al., 2011). Growing of resistant cultivars is the safest and economically profitable way to control fungal plant diseases. However, wheat breeding for resistance is complicated because of the pathogen's ability to overcome the host resistance. The new pathotypes are continuously emerging due to sexual recombination and mutational processes. The nature of urediniospores enables them to migrate by air for thousands of kilometers, which causes the spread of new virulent pathotypes throughout the world (Kolmer, 2005). The information about the effectiveness of *Lr*-genes and also the consequent monitoring of virulence dynamics are necessary for successful wheat breeding for resistance to leaf rust and for timely detection of new phenotypes to adjust the breeding programs.

A nomenclature to divide the population of leaf rust into separate pathotype "races" was proposed after discovery of physiologic races by Mains and Jackson in 1926 (Mains and Jackson, 1926). Then, after a few modifications, the nomenclature was adopted on the basis of eight cultivars (Johnson and Browder, 1966). However, after emergence of new pathotypes which, according to their characteristics, did not match up with this code, different researchers assigned them different designations. Another disadvantage is that these differentials carry *Lr*-genes in different genetic background and some of them carry several *Lr*-genes, for example 'Carina' (*Lr2b*, *LrB*), 'Brevit' (*Lr2c*, *LrB*), 'Webster' (*Lr2a*, *Lr14a*, *Lr27*), 'Loros' (*Lr2c*, *Lr2d*), 'Mediterranean' (*Lr2a*, *Lr3a*) share different *Lr*-genes (McIntosh et al., 1995; GRIS, 2014). When the near-isogenic lines which possess single *Lr*-genes were developed they started to be used as differentials among new set of wheat cultivars. Most researchers have replaced the term physiologic race by the term pathotype or phenotype (Kolmer, 2005; Hanzalova and Bartos, 2006). In 1989, a North American System of Nomenclature for *Puccinia triticina* was proposed. According to this differential set, it was proposed to use 12 near-isogenic lines of 'Thatcher' with single *Lr*-

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genes. The differentials were grouped into three sets of four lines. The first set of differentials consisted of 'Thatcher' lines with genes *Lr1*, *Lr2a*, *Lr2c*, *Lr3a*, the second *Lr9*, *Lr16*, *Lr24*, *Lr26*, the third *Lr3ka*, *Lr11*, *Lr17a*, *Lr30*. The pathotypes characteristics are coded in accordance with the type of reaction by one of 16 letters for each of three sets; accordingly, a three-letter code was assigned to the pathotypes (Long and Kolmer, 1989). This nomenclature has become the most common and is being extensively used throughout most of the world (Huerta-Espino et al., 2011). Virulence analysis of leaf rust population requires information about changes of virulence first of all for genes which present interest for specific climatic-territorial areas where the cultivars are planned to be grown. Therefore phytopathologists are attempting to add the differentials with certain *Lr*-genes to the differential set, according to specificity of pathogen's population of their region. In the last years, a series of additional sets of 'Thatcher' near-isogenic lines has been proposed to be included in the original North American differential set in the USA and other countries (Singh, 1991; Kolmer and Liu, 2000; McVey et al., 2004; Mantovani et al., 2010). Supplemental fourth set of *LrB*, *Lr10*, *Lr14a*, and *Lr18* was added by Kolmer et al. (2007) and included in the national virulence surveys in the USA and Canada. The fifth set of differentials with two 'Thatcher' lines with *Lr21*, *Lr28* and two winter wheat lines with genes *Lr41* and *Lr42* were added to the national virulence surveys in the USA in 2004, but in 2007 the line with *Lr42* was dropped after it had been determined that it also possessed *Lr24* (Kolmer et al., 2007; Kolmer and Hughes, 2013).

In European countries, a standard differential set which consists of 15 Thatcher near isogenic lines with the single leaf rust genes *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr9*, *Lr11*, *Lr15*, *Lr17*, *Lr19*, *Lr21*, *Lr23*, *Lr24*, *Lr26* and *Lr28* has been adopted. Pathotype analysis is conducted using digital nomenclature with triplet set and octal codes of virulence according to Hanzalova and Bartos (2006) and to Goyeau et al. (2006). Researchers in Nepal, Bangladesh, Pakistan, South Africa, and others countries have reported races using both their own nomenclatures and the North American Nomenclature. However, breeders from India and Australia are still using their own binomial systems of nomenclature (Huerta-Espino et al., 2011; Terefe et al., 2014).

Physiologic races of *P. triticina* have been studied in Plant Breeding and Genetics Institute in Ukraine since the 1960's on an old standard differential set. In the present study, we used both the old standard differential set and the North American Nomenclature to compare the old findings of the old standard differential set with the North American differential set for *P. triticina*. The aim of the study was to conduct a pathotype analysis of leaf rust population and to study the effectiveness of 53 known *Lr*-genes at the seedling and adult plant stages in the south of Ukraine.

MATERIAL AND METHODS

Samples of leaf rust infected wheat leaves of different cultivars and breeding lines were collected from commercial fields and research plots at various locations throughout the south of Ukraine. All collected leaves were air dried at room temperature and stored at 4 °C until spores were collected for inoculation and multiplication of the inoculum. The virulence survey was based on monopustule isolates. *Puccinia triticina* urediniospores from individual collections were scraped off and mixed with water and Tween 20. The susceptible cultivars 'Michigan Amber' and 'Odesskaya polukarlikovaya' were inoculated with the suspension of urediniospores. Approximately 14 d after inoculation, using a microbiological loop, separate monopustule isolates were transferred on the detached leaves of 'Michigan Amber' and 'Odesskaya polukarlikovaya', kept in the solution of benzimidazol in Petri dishes (Babayants and Babayants, 2014). Microbiological loop was sterilized each time before transferring the new pustules. Each monopustule isolate was transferred into a separate Petri dish. When the inoculum was multiplied, approximately after 14 d, differentials were also inoculated using the same procedure.

A total of 113 monopustule isolates for phenotype and virulence analyses were studied. Phenotype analysis was conducted on the standard differential set of eight cultivars: Malakoff, Carina, Brevit, Webster, Loros, Mediterranean, Hussar, Democrat (Johnson and Browder, 1966) and the North American differential set for *P. triticina* on 16 near-isogenic lines of 'Thatcher' with single leaf rust resistance genes *Lr1*, *Lr2a*, *Lr2c*, *Lr3a*, *Lr3ka*, *Lr9*, *Lr10*, *Lr11*, *Lr14a*, *Lr16*, *Lr17a*, *Lr18*, *Lr24*, *Lr26*, *Lr30*, and *LrB* (Long and Kolmer, 1989; Kolmer et al., 2007).

Virulence analysis of leaf rust population was studied on the near-isogenic lines of 'Thatcher' with single genes: *LrB*, *Lr1*, *Lr2a*, *Lr2c*, *Lr3a*, *Lr3ka*, *Lr9*, *Lr10*, *Lr11*, *Lr14a*, *Lr17*, *Lr18*, *Lr19*, *Lr20*, *Lr21*, *Lr22a*, *Lr23*, *Lr24*, *Lr25*, *Lr26*, *Lr29*, *Lr30*, *Lr38*, and *Lr64*. In addition four cultivars/lines 'Pavon 76' (*Lr1*, *Lr10*, *Lr13*, *Lr14a*, *Lr2b*, *Lr22a*, *Lr27*, *Lr46*), 'Pavon 753' (*Lr47*), 'KS86WGRC02' (*Lr39*) and 'KS92WGRC16' (*Lr42*, *Lr39*, *Lr21*) were used.

The effectiveness of 53 *Lr*-genes was studied both at the seedling stage and at the stage of adult plant. The resistance at the seedling stage was assessed in the greenhouse under controlled conditions (temperature: +20 ± 2 °C, illuminance: 10 000 lux, 16:8 h photoperiod). Ten days old plants were inoculated by a mix of urediniospores with talcum powder. After inoculation, to create the conditions of a dew chamber, the plants were put in the polyethylene bags and stored in the dark at 21 °C for 16 h. Type of resistance and disease severity were scored on the 12th day after inoculation. Type of resistance

at the seedling stage was scored according to the scale: very resistant (VR), resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S), and very susceptible (VS). Disease severity was scored by the scale of Peterson (Babayants and Babayants, 2014).

The field resistance was studied in the artificially infected nursery. The differentials were grown in 1 m long rows (three rows for each differential), the distance between rows was 30 cm. Two rows of the most rust-susceptible spreader ('Odesskaya polukarlikovaya') were planted around the nursery. Also, one row of this susceptible control was planted at every 10th test entry. The leaf rust differentials were inoculated with a mixture of urediniospores and talcum powder according to Babayants procedure (Babayants and Babayants, 2014). Inoculation was done in 22 April 2013 and 28 April 2014. Environmental conditions for pathogen development are presented in Table 1. Adult resistance in the field was scored according to a 9 point scale: 1-2 very high susceptibility, 3 high susceptibility, 4 susceptibility, 5 moderate susceptibility, 6 moderate resistance, 7 resistance, 8 high resistance, and 9 immunity (Babayants and Babayants, 2014). The seeds of differentials and wheat lines/cultivars with *Lr*-genes were provided via USDA Germplasm Resources Information Network (GRIN) (USDA ARS, 2014).

Table 1. Meteorological data during the inoculation and *Puccinia triticina* development.

Year, Month	Decade	Precipitation	Relative humidity	Air temperature		
				Max	Min	Avg
		mm	%		ºC	
2013						
	1	2.3	80	22.2	2.5	8.0
April	2	36.1	75	22.3	1.8	9.8
	3	0.7	64	27.6	5.0	15.7
	1	0.0	68	29.2	11.5	18.7
May	2	4.0	77	28.7	11.4	19.2
	3	0.8	81	29.4	11.5	19.3
	1	29.1	84	28.0	12.0	19.0
June	2	54.4	70	32.0	14.9	22.8
	3	41.2	73	34.2	14.3	24.0
2014						
	1	-	68	22.6	-2.3	8.6
April	2	4.6	82	23.6	5.0	10.8
	3	-	70	23.4	5.3	13.9
	1	9.8	75	22.7	3.3	13.7
May	2	11.6	78	25.7	9.9	16.3
	3	4.8	69	32.7	13.3	21.3
	1	46.1	67	34.0	10.3	21.6
June	2	14.0	62	30.0	12.3	20.7
	3	7.5	69	29.0	10.4	20.0

Max: maximum, Min: minimum, Avg: average.

RESULTS

Phenotype composition

Phenotype analysis was conducted using two differential sets – the standard differential set and the North American System of Nomenclature for *P. triticina*. On the standard differential set, 12 different phenotypes were revealed in 2013. The most common races were 77 (75%) and 144 (6%), noticed also the others 149 (4%), 117 (3%), 192 (2%), 114 (2%), 6 (2%), 21 (2%), 42 (1%), 57 (1%), 20 (1%), 122 (1%) (Table 2). Forty phenotypes for *P. triticina* were identified on the North American Nomenclature. Among them two phenotypes TGTT (24%) and TJTT (8%) were dominant. Phenotypes PHTT, TQTT, TGPT, RGTT, TGKT, and PBKK had an occurrence frequency of 3% each, while others were represented by single isolates. Phenotype TGTT was avirulent to *Lr9*, *Lr24* and *Lr26* and it was virulent against the other 13 *Lr*-genes of the differential set. TJTT was avirulent only to *Lr9* and *Lr26*. Phenotypes TRTT and TSTT with the frequency of occurrence of 1.5% possess the broadest spectrum of virulence, the former was avirulent only to *Lr24* and the latter to *Lr26*. A total of 40 phenotypes of wheat leaf rust were identified on the North America Nomenclature in the south of Ukraine in 2013 (Table 3).

Virulence analysis of *Puccinia triticina* population

The virulence analysis was done on 26 near isogenic lines of 'Thatcher' and also on the four wheat cultivars/lines (Tables 4 and 5). None of the 113 studied monopustule isolates had virulence to gene *Lr19*. In the previous years the frequency of virulence to *Lr19* varied from 0% to 4% (Babayants et al., 2004; Babayants, 2011).

Table 2. The virulence formula and frequency of *Puccinia triticina* phenotypes identified on the standard differential set in the south of Ukraine in 2013.

Race	Malakof	Carina	Brevit	Webster	Loros	Mediterranean	Hussar	Democrat	%
77	S	S	S	S	S	S	S	S	75
144	S	S	S	R	S	S	S	S	6
149	S	R	S	S	S	S	S	S	4
117	S	S	S	S	S	R	S	S	3
192	S	S	S	S	R	S	S	S	2
114	S	S	S	I	S	S	R	S	2
6	S	R	S	R	S	S	S	S	2
21	S	S	R	S	S	S	S	S	2
42	S	I	S	S	S	S	S	S	1
57	R	S	S	S	S	S	S	S	1
122	S	S	S	S	S	S	R	S	1
20	S	S	S	S	S	R	S	R	1
Total									100

R: Resistant, S: susceptible, I: intermediate.

Table 3. Virulence formula and frequency of *Puccinia triticina* phenotypes in the south of Ukraine in 2013 identified on the North American Nomenclature (16 near-isogenic lines with single *Lr*-genes).

Phenotype	Avirulence/virulence formula	Pcs.	%
TGTT	*Lr9, Lr24, Lr26/Lr1, Lr2a, Lr2c, Lr3a, Lr16, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	16	24
TJTT	*Lr9, Lr26/Lr1, Lr2a, Lr2c, Lr3a, Lr16, Lr24, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	5	8
PHTT	*Lr2a, Lr9, Lr24/Lr1, Lr2c, Lr3a, Lr16, Lr26, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	2	3
TQTT	*Lr24, Lr26/Lr1, Lr2a, Lr2c, Lr3a, Lr9, Lr16, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	2	3
TGPT	*Lr9, Lr24, Lr11/Lr1, Lr2a, Lr2c, Lr3a, Lr16, Lr26, Lr3ka, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	2	3
TGKT	*Lr9, Lr24, Lr26, Lr3ka/Lr1, Lr2a, Lr2c, Lr3a, Lr16, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	2	3
RJTT	*Lr2c, Lr9, Lr26/Lr1, Lr2a, Lr3a, Lr16, Lr24, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	2	3
RGTT	*Lr2c, Lr9, Lr24, Lr26/Lr1, Lr3a, Lr16, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	2	3
PBKK	*Lr2a, Lr9, Lr16, Lr24, Lr26, Lr3ka, LrB/Lr1, Lr2c, Lr3a, Lr11, Lr17a, Lr30, Lr10, Lr14a, Lr18*	2	3
TRTT	*Lr24/Lr1, Lr2a, Lr2c, Lr3a, Lr9, Lr16, Lr26, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
TSTT	*Lr26/Lr1, Lr2a, Lr2c, Lr3a, Lr9, Lr16, Lr24, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
THTT	*Lr9, Lr24/Lr1, Lr2a, Lr2c, Lr3a, Lr16, Lr26, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
TQSP	*Lr24, Lr26, Lr30, Lr10/Lr1, Lr2a, Lr2c, Lr3a, Lr9, Lr16, Lr3ka, Lr11, Lr17a, LrB, Lr14a, Lr18*	1	1.5
TQQN	*Lr24, Lr26, Lr17a, Lr30, Lr10, Lr18/Lr1, Lr2a, Lr2c, Lr3a, Lr9, Lr16, Lr3ka, Lr11, LrB, Lr14a*	1	1.5
TNTT	*Lr16, Lr26/Lr1, Lr2a, Lr2c, Lr3a, Lr9, Lr24, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
TJKT	*Lr9, Lr26, Lr3ka/Lr1, Lr2a, Lr2c, Lr3a, Lr16, Lr24, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
TGTM	*Lr9, Lr24, Lr26, Lr10, Lr14a/Lr1, Lr2a, Lr2c, Lr3a, Lr16, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr18*	1	1.5
TGQM	*Lr9, Lr24, Lr26, Lr17a, Lr30, Lr10, Lr14a/Lr1, Lr2a, Lr2c, Lr3a, Lr16, Lr3ka, Lr11, LrB, Lr18*	1	1.5
TGQT	*Lr9, Lr24, Lr26, Lr17a, Lr30/Lr1, Lr2a, Lr2c, Lr3a, Lr16, Lr3ka, Lr11, LrB, Lr10, Lr14a, Lr18*	1	1.5
TDQL	*Lr9, Lr16, Lr26, Lr17a, Lr30, Lr10, Lr14a, Lr18/Lr1, Lr2a, Lr2c, Lr3a, Lr24, Lr3ka, Lr11, LrB*	1	1.5
TBTT	*Lr9, Lr16, Lr24, Lr26/Lr1, Lr2a, Lr2c, Lr3a, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
RGHT	*Lr2c, Lr9, Lr24, Lr26, Lr3ka, Lr17a/Lr1, Lr2a, Lr3a, Lr16, Lr11, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
RHTT	*Lr2c, Lr9, Lr24/Lr1, Lr2a, Lr3a, Lr16, Lr26, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
RKTT	*Lr2c, Lr9/Lr1, Lr2a, Lr3a, Lr16, Lr24, Lr26, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
QGMS	*Lr2c, Lr3a, Lr9, Lr24, Lr26, Lr11, Lr17a, Lr18/Lr1, Lr2a, Lr16, Lr3ka, Lr30, LrB, Lr10, Lr14a*	1	1.5
PQFT	*Lr2a, Lr24, Lr26, Lr3ka, Lr11/Lr1, Lr2c, Lr3a, Lr9, Lr16, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
PKTT	*Lr2a, Lr9/Lr1, Lr2c, Lr3a, Lr16, Lr24, Lr26, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
PJTT	*Lr2a, Lr9, Lr26/Lr1, Lr2c, Lr3a, Lr16, Lr24, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
PHCT	*Lr2a, Lr9, Lr24, Lr3ka, Lr11, Lr17a/Lr1, Lr2c, Lr3a, Lr16, Lr26, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
PGTT	*Lr2a, Lr9, Lr24, Lr26/Lr1, Lr2c, Lr3a, Lr16, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
PGFT	*Lr2a, Lr9, Lr24, Lr26, Lr3ka, Lr11/Lr1, Lr2c, Lr3a, Lr16, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
NGCS	*Lr2a, Lr3a, Lr9, Lr24, Lr26, Lr3ka, Lr11, Lr17a, Lr18/Lr1, Lr2c, Lr16, Lr30, LrB, Lr10, Lr14a*	1	1.5
NGKT	*Lr2a, Lr3a, Lr9, Lr24, Lr26, Lr3ka/Lr1, Lr2c, Lr16, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
NHTT	*Lr2a, Lr3a, Lr9, Lr24/Lr1, Lr2c, Lr16, Lr26, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
KSTT	*Lr1, Lr26/Lr2a, Lr2c, Lr3a, Lr9, Lr16, Lr24, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
KJTT	*Lr1, Lr9, Lr26/Lr2a, Lr2c, Lr3a, Lr16, Lr24, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
KGTT	*Lr1, Lr9, Lr24, Lr26/Lr2a, Lr2c, Lr3a, Lr16, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
HGTT	*Lr1, Lr2c, Lr9, Lr24, Lr26/Lr2a, Lr3a, Lr16, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
FKTT	*Lr1, Lr2a, Lr9/Lr2c, Lr3a, Lr16, Lr24, Lr26, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
FGTT	*Lr1, Lr2a, Lr9, Lr24, Lr26/Lr2c, Lr3a, Lr16, Lr3ka, Lr11, Lr17a, Lr30, LrB, Lr10, Lr14a, Lr18*	1	1.5
Total		66	100

The frequency of monopustule isolates virulent to *Lr9* was 13%, which is substantially higher than that over the last 20 yr, the maximal frequency of virulence (10%) was noticed in 2003 (Babayants, 2011). The virulence to *Lr29* was 11% lower than that in previous years. Gene *Lr64* was studied for the first time, the frequency of virulent monopustule isolates to it was 19%. In comparison with previous years the frequency of virulence to *Lr26* slightly decreased (17%), in previous years it varied from 40% to 80% (Babayants, 2011). The frequency of virulent monopustule isolates to gene *Lr24* was 26%, in previous years it varied from low (less than 10%) in 1996, 1998, 2002, 2006, 2007 to high in 1999, 2001, and 2003 (> 50%) (Babayants, 2011).

The frequency of virulence to *Lr25* was 26% (Table 4). Medium frequency of virulence was observed against genes *Lr2a*, *Lr21*, *Lr22a* and *Lr23*. A high frequency of virulence was noticed to genes *LrB*, *Lr1*, *Lr2c*, *Lr3a*, *Lr3ka*, *Lr10*, *Lr11*, *Lr14a*, *Lr16*, *Lr17*, *Lr18*, *Lr20* and *Lr30* (Table 4). Virulence to lines KS86WGRC02 (*Lr39/41*), KS92WGRC16 (*Lr42*, *Lr39*, *Lr21*) and 'Pavon 753' (*Lr47*) was 70%, 54%, and 21% respectively, to 'Pavon 76' (*Lr1*, *Lr10*, *Lr13*, *Lr14a*, *Lr2b*, *Lr22a*, *Lr27*, *Lr46*) was 96% (Table 5).

Effectiveness of the *Lr*-genes in the seedling stage

Seedling resistance of *Lr*-genes was studied after artificial inoculation by local population of leaf rust. The effectiveness of genes was divided according to the type of reaction into the groups VR, R, MS, S, and VS. The genes within each group were subdivided also according to the disease severity (Table 6).

Highly efficient were *Lr9* and *Lr19*, disease symptoms were not observed. Genes *Lr29* and *Lr47* also had resistance type of reaction, but the disease severity was 5% and 25%, respectively (Table 6). Moderately susceptible were genes *Lr24* and *Lr25* with a disease severity of 5%, this group also included genes *Lr56* with a disease severity of 10%, 15% for *Lr64*, *Lr39* and *Lr42*, 25% for *Lr45*, 40% for *Lr15, Lr16*, *Lr51*, and *Lr52*, 65% for *Lr44* and *Lr50* (Table 6). The greater part of genes fell within the group exhibiting susceptibility. Lines carrying genes *Lr23*, *Lr22a* showed lower level of susceptibility (5%) in this group, a bit higher level of susceptibility (10%) was demonstrated by lines with *Lr18*, *Lr21* and *Lr26* genes (Table 6). Lines with genes *Lr1*, *Lr3bg* and 'Odesskaya polukarlikovaya' were highly susceptible with a susceptibility level of 65% (Table 6).

Table 4. Frequency of occurrence of virulent isolates of *Puccinia triticina* in the south of Ukraine in 2013 (at the seedling stage).

Gene	Accession number[1]	Pedigree[2]	Frequency of virulence, %
LrB	GSTR 446	Thatcher*6/Brevit	97
Lr1	GSTR 402	Thatcher*6/Centenario	91
Lr2a	GSTR 403	Thatcher*6/Webster	78
Lr2c	GSTR 405	Thatcher*6/Brevit	86
Lr3a	GSTR 406	Thatcher*6/Democrat	94
Lr3ka	GSTR 408	Thatcher*6/Klein Aniversario	84
Lr9	GSTR 409	Thatcher*6/*Aegilops umbellulata*	13
Lr10	GSTR 410	Thatcher*6/Lee	92
Lr11	GSTR 411	Thatcher*6/Hussar	89
Lr14a	GSTR 414	Thatcher*6/Hope	95
Lr16	GSTR 417	Thatcher*6/Exchange	93
Lr17	GSTR 418	Thatcher*6/Klein Lucero	88
Lr18	GSTR 419	Thatcher*6/Africa 43	94
Lr19	GSTR 420	Thatcher*6/*Agropyron elongatum*	0
Lr20	GSTR 421	Thatcher*6/Thew	81
Lr21	GSTR 422	Thatcher*6/*Aegilops tauschii*	68
Lr22a	GSTR 423	Thatcher*6/*Aegilops tauschii*	78
Lr23	GSTR 424	Thatcher*6/Gabo	66
Lr24	GSTR 425	Thatcher*6/*Agropyron elongatum*	26
Lr25	GSTR 426	Thatcher*6/Rosen (rye)	45
Lr26	GSTR 427	Thatcher*6/Imperial (rye)	18
Lr29	GSTR 429	Thatcher*6/*Agropyron elongatum*	11
Lr30	GSTR 430	Thatcher*6/Terenzio	92
Lr64	GSTR 445	Thatcher*6/*Triticum dicoccoides*	19

[1]Accession numbers in accordance with Research Service of Germplasm Resource Information Network (GRIN).
[2]Pedigree in accordance with Genetic Resources Information System for Wheat and Triticale (GRIS).

Table 5. Frequency of occurrence of virulent monopustule isolates of *Puccinia triticina* in cultivars/lines with *Lr*-genes in the south of Ukraine in 2013.

№ п/п	Cultivar, line	*Lr*-gene[1]	Accession number[2]	%
1	KS86WGRC02	*Lr39*	PI 504517	70
2	KS92WGRC16	*Lr42, Lr39, Lr21*	PI 592728	54
3	Pavon 753	*Lr47*	GSTR 440	21
4	Pavon 76	*Lr1, Lr10, Lr13, Lr14a, Lr2b, Lr22a, Lr27, Lr46*	PI 519847	96

[1]The presence of *Lr*-genes in accordance with Genetic Resources Information System for Wheat and Triticale (GRIS).
[2]Accession numbers in accordance with Research Service of Germplasm Resource Information Network (GRIN).

Table 6. Results of phytopathological evaluation of *Lr*-genes at the seedling stage.

Type of infection	Disease severity	Gene, cultivar
VR	0	*Lr9, Lr19*
R	5	*Lr29*
	25	*Lr47*
MS	5	*Lr24, Lr25*
	10	*Lr56*
	15	*Lr64, Lr39, Lr42*
	25	*Lr45*
	40	*Lr15, Lr16, Lr51, Lr52*
	65	*Lr44, Lr50*
S	5	*Lr23, Lr22a*
	10	*Lr18, Lr21, Lr26*
	15	*Lr3ka, Lr20, Lr45*
	25	*Lr35, Lr46, Lr54, Lr63*
	40	*Lr2a, Lr2c, Lr10, Lr11, Lr12, Lr17, Lr27, Lr30, Lr32, Lr34, Lr36, Lr37, Lr60*
	65	*Lr2b, Lr3, Lr13, Lr14a, Lr14b, Lr28, Lr33, Lr38, Lr53*
VS	65	*Lr1, Lr3bg,* 'Odesskaya polukarlikovaya'

Effectiveness of the *Lr*-genes in the field

Phytopathological evaluation of wheat lines and cultivars with known genes of resistance to *P. triticina* was observed in the infected nurseries in 2013 and 2014 (Table 7).

Environmental conditions during pathogen inoculation were more favorable in 2013, indicator of disease development 'Odesskaya polukarlikovaya' scored 2 points. In 2014, there was observed a slightly lower infection pressure of the pathogen, 'Odesskaya polukarlikovaya' scored 4 points. Genes *Lr9*, *Lr19*, *Lr24*, *Lr29*, *Lr42*, *Lr47*, *Lr50* and *Lr51* were resistant in the field tests during the two experimental years. The genes *Lr25*, *Lr44*, *Lr52*, *Lr53*, *Lr56* and *Lr64* were resistant in 2014, but in 2013 they were susceptible (Table 7).

Table 7. Results of phytopathological evaluation of *Lr*-genes at the adult plant stage.

Gene	Status/Name	Accession number[1]	2013[2]	2014[2]
Lr1	NIL	GSTR 402	2	4
Lr2a	NIL	GSTR 403	2	4
Lr2b	NIL	GSTR 404	2	4
Lr2c	NIL	GSTR 405	2	3
Lr3	NIL	GSTR 406	2	2
Lr3bg	NIL	GSTR 407	2	4
Lr3ka	NIL	GSTR 408	2	5
Lr9	NIL	GSTR 409	8	7
Lr10	NIL	GSTR 410	2	4
Lr11	NIL	GSTR 411	2	4
Lr12	NIL	GSTR 412	6	5
Lr13	NIL	GSTR 413	2	5
Lr14a	NIL	GSTR 414	2	3
Lr14b	NIL	GSTR 415	2	4
Lr15	NIL	GSTR 416	4	5
Lr16	NIL	GSTR 417	2	5
Lr17	NIL	GSTR 418	5	5
Lr18	NIL	GSTR 419	5	5
Lr19	NIL	GSTR 420	9	7
Lr20	NIL	GSTR 421	2	6
Lr21	NIL	GSTR 422	5	4
Lr22a	NIL	GSTR 423	6	4
Lr23	NIL	GSTR 424	6	5
Lr24	NIL	GSTR 425	8	7
Lr25	NIL	GSTR 426	5	8
Lr26	NIL	GSTR 427	4	6
Lr27	Gatcher	PI 377884	3	5
Lr28	NIL	GSTR 428	4	5
Lr29	NIL	GSTR 429	7	8
Lr30	NIL	GSTR 430	2	5
Lr32	NIL	GSTR 431	4	5
Lr33	NIL	GSTR 432	2	3
Lr34	NIL	GSTR 433	5	5
Lr35	NIL	GSTR 434	5	5
Lr36	Genetic material	GSTR 435	6	6
Lr37	NIL	GSTR 436	5	6
Lr38	NIL	GSTR 437	2	4
Lr39/41	KS86WGRC02	PI 504517	6	7
Lr42	KS92WGRC16	PI 592728	9	8
Lr44	NIL	GSTR 438	4	8
Lr45	NIL	GSTR 439	6	5
Lr46	Pavon 76	PI 519847	6	6
Lr47	Pavon 753	GSTR 440	8	8
Lr50	KS96WGRC36	PI 604221	9	8
Lr51	NIL	GSTR 441	8	8
Lr52	NIL	GSTR 442	6	8
Lr53	98M71	PI 648417	6	8
Lr54	Genetic material	PI 648418	3	6
Lr56	Genetic material	PI 648419	8	8
Lr60	Genetic material	GSTR 443	3	4
Lr63	NIL	GSTR 444	2	4
Lr64	NIL	GSTR 445	6	8
'Odesskaya polukarlikovaya' indicator of disease development			2	4

[1]Accession numbers in accordance with Research Service of Germplasm Resource Information Network (GRIN).
[2]Scoring by 1 to 9 point scale, 1: very high susceptibility, 9: immunity.

DISCUSSION

Twelve phenotypes were identified on the old differential set in the population of leaf rust in 2013. The most common phenotypes were 77 (75%) and 144 (6%). Over the last 20 yr a total of 47 known and 15 new races in the population of *P. triticina* were found in the south of Ukraine (Babayants, 2011). According to the North American nomenclature, the dominant phenotypes in the population of leaf rust were TGTT (24%) and TJTT (8%), both phenotypes possess a broad spectrum of virulence and according to the old nomenclature both may belong to the "race 77". However, the first phenotype was avirulent to *Lr24*, whereas the second phenotype was virulent to it. The obtained results showed that the North American differential set provides more important information and much better differential ability than the old standard differential set used before in Ukraine. The presence of the effective gene *Lr9* and genes with partial resistance *Lr24* and *Lr26* in the nomenclature makes more significant phenotype differentiation for south of Ukraine. The advantage of the North American nomenclature over the standard differential set also was established by other researchers (Todorova and Kiryakova, 2001). Moreover, the researchers who work independently using the letter code nomenclature will give the same code-name to any new physiologic pathotypes that code the same on this system. This allows researchers to speak the same language and have a common understanding of the virulence phenotypes. However, some *Lr*-genes which are effective in the south of Ukraine are not included in the differential set. Therefore for virulence analysis we used additional 'Thatcher' near-isogenic lines with *Lr19*, *Lr20*, *Lr21*, *Lr22a*, *Lr23*, *Lr24*, *Lr25*, *Lr29* and *Lr64* *Lr*-genes, 'Pavon 76' (*Lr1*, *Lr10*, *Lr13*, *Lr14a*, *Lr2b*, *Lr22a*, *Lr27*, *Lr46*) and wheat lines 'Pavon 753' (*Lr47*), KS86WGRC02 (*Lr39*) and KS92WGRC16 (*Lr42*, *Lr39*, *Lr21*) which are new or possess resistance and present local interest for our environments. Virulence analysis exhibited that the frequency of virulence to *Lr9* was increased by 13% in 2013. It is generally accepted, that when the frequency of virulence reaches higher than 10% efficiency of gene may be overcome by pathogen. When the frequency of virulent pathotypes and/or their aggressiveness increase, this gene may be shortly overcome by the pathogen in the south of Ukraine. Breaking down of *Lr9* was noticed in the USA and Canada (McCallum et al., 2011; Kolmer et al., 2012).

Lr19 remains highly efficient, no virulent isolates were found in the population of leaf rust. Virulence to that resistant gene has not been found in other European countries (Mesterházy et al., 2000; Huerta-Espino et al., 2011). But according to the published data a large quantity of virulent pathotypes (40%) were detected in the population of leaf rust in Volga region of Russia (Kurbanova, 2011; Ivanova, 2013).

The gene *Lr24* derived from *Agropyron elongatum* (Host.) Neviski (McIntosh et al., 2013). According to the last monitoring, virulence to *Lr24* was very rare in Germany, Spain, Hungary, Slovak Republic (Huerta-Espino et al., 2011), and Czech Republic (Hanzalova et al., 2013), Lithuania (Liatukas, 2003) and China (Liu and Chen, 2012). In Ukraine, *Lr24* exhibited moderate susceptibility at the seedling stage and resistance at adult plant stages.

Gene *Lr29* derived from *A. elongatum* (McIntosh et al., 2013) under artificial inoculation by population of leaf rust at the seedling stage provided resistance; the frequency of virulence to it was 11%. However, in the previous years virulence to it was relatively high. This indicates that this gene is unreliable against leaf rust (Babayants et al., 2004; Babayants, 2011).

Slow rusting gene *Lr34* has been providing resistance for more than 50 yr, and *Lr46* has remained effective since 1976 in the USA. Also, it was established that *Lr34* has the ability to enhance the resistance to *P. triticina* in combination with other *Lr*-genes and to provide positive pleiotropic effect to other wheat diseases (German and Kolmer, 1992; Kolmer et al., 2008). The synergy between *Lr34* and other *Lr*-genes also was detected in our environments (unpublished data). However, *Lr34* in single use did not provide sufficient level of resistance in the population of southern Ukraine. It was highly susceptible at the seedling stage and susceptible (scored 6) at the adult plant stages. Gene *Lr46* in combination with other genes *Lr1*, *Lr10*, *Lr13*, *Lr14a*, *Lr2b*, *Lr22a*, and *Lr27* in background of 'Pavon 76' also did not provide sufficient level of resistance.

Gene *Lr42* derived from *Aegilops tauschii* Coss. (McIntosh et al., 2013) exhibited moderate susceptibility in the seedling test and resistance in the field test. This indicates that it provides sufficient level of resistance only at the adult plant stages. Gene *Lr47* derived from *Triticum speltoides* (Tausch) Gren. ex K. Richt. (McIntosh et al., 2013) provided resistance in seedling test and in the field in 2013 and 2014, but virulence to it at the seedling plant stage was 21%. This indicates that resistance may be soon overcome by pathogen. Genes *Lr50*, *Lr51* and *Lr56* were studied by us for the first time and exhibited resistance in the field test during 2 yr, but *Lr50* was susceptible, and *Lr51*, *Lr56* were moderately susceptible in the seedling test. That may indicate that they act as the adult plant stages genes. Gene *Lr64* derived from *Triticum dicoccoides* (McIntosh et al., 2013) was also studied by us for the first time. At the seedling stage plants showed moderate susceptibility, the frequency of virulence was 19%, at the adult plant stages this gene provided susceptibility in 2013 and resistance in 2014. The resistance in 2014 and susceptibility in 2013 of genes *Lr25*, *Lr44*, *Lr52*, *Lr53*, *Lr56* and *Lr64* in the field test can be caused by different pathogen development. Artificial leaf rust inoculation was more successful in 2013, which can be explained by a very small amount of precipitation in April 2014 during the crucial period for inoculation (Table 1). Urediniospores

begin to develop a germ tube and penetrate the cell only when the moisture is present in the form of dew or light rain on the leaf surface. Germination occurs after 8 h at 18 °C, spores possess the ability to retain viability only 1-3 d after inoculation under field conditions in the absence of the dew period (Babayants and Babayants, 2014). Due the better environment conditions for pathogen development, the infection of leaf rust developed better in 2013 than in 2014. In 2013, when infection pressure was higher genes *Lr25*, *Lr44*, *Lr52*, *Lr53*, *Lr56*, and *Lr64* were susceptible, which may indicate their insufficiency; under optimal conditions for pathogen development they cannot provide sufficient level of resistance.

The Ukrainian population of leaf rust consists of broad range of pathotypes with different spectrum and frequency of virulence. This point to high pathogen evolutionary ability, and consequently a large part of known *Lr*-genes have lost their efficiency and cannot be used as donors of resistance. Thus, single use of these *Lr*-genes cannot provide durable defense against wheat leaf rust. Theoretically, if resistance is controlled at one single locus, only one mutation in the corresponding avirulent gene may lead to emergence of a new virulent pathotype. Therefore several breeding strategies, pyramiding and slow rusting to enhance the durability of resistance to leaf rust have been proposed. Gene pyramiding is incorporation of several *Lr*-genes into a single genotype. Slow rusting is conferred by genes which provide a longer latent period of disease, lower spore production and as a result smaller areas under the disease progress curve than a susceptible control (Singh et al., 2011). For gene pyramiding it is needed to choose the major genes to which corresponds a very low ratio of virulence in pathogen population and then they could be used in combination with seedling partial resistance genes or in combination with adult non-specific genes (Krattinger et al., 2009; Dakouri et al., 2013).

CONCLUSIONS

The population of leaf rust consisted of different phenotypes in the south of Ukraine. According to the standard differential set, there were identified 12 phenotypes of which the most common were 77 (75%) and 144 (6%), 149 (4%), 117 (3%), 192 (2%), 114 (2%), 6 (2%), 21 (2%), 42 (1%), 57 (1%), 20 (1%), 122 (1%). The North American nomenclature provides much more differential ability, and using this nomenclature we identified 40 phenotypes, of which the most frequent were TGTT (24%) and TJTT (8%), phenotypes TRTT (1.5%) and TSTT (1.5%) possess the broadest spectrum of virulence among the isolates found in the south of Ukraine. Among all studied *Lr*-genes no isolates were virulent to *Lr19*, against other *Lr*-genes virulence frequency varied from 11 to 97%. Low frequency of virulence was observed to *Lr29* (11%), *Lr9* (13%) and *Lr47* (21%), high level of virulence was detected to other genes. Most of the known *Lr*-genes do not provide resistance either at the seedling or at the adult plant stage. Genes *Lr9*, *Lr19*, *Lr29* and *Lr47* were highly effective both at the seedling stage and in the field test. Genes *Lr24*, *Lr42*, *Lr50*, *Lr51* and *Lr56* were effective only at the adult plant stage during the two experimental years.

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