The Virulence Spectrum of the Wheat Leaf Rust Population Analyzed in the Czech Republic from 2002 to 2011

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Abstract

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The research report presents a summary of wheat leaf rust virulence surveys in the Czech Republic from 2002 to 2011. Determination of virulence was based on infection types on Thatcher near-isogenic lines (NILs) with the resistance genes *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr9*, *Lr10*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr19*, *Lr21*, *Lr23*, *Lr24*, *Lr26* and *Lr28*, respectively. Virulence for *Lr9* and *Lr 19* occurred only sporadically in the investigated period. On average, virulence for *Lr2a*, *Lr2b*, *Lr24* and *Lr28* was low. The highest frequency of virulence was found for *Lr3a*, *Lr10*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23* and *Lr26*. During the investigation period we recorded a shift from prevailing virulence for *Lr2c* and avirulence for *Lr1* to avirulence for a*Lr2c* and virulence for *Lr1*. Ther virulences in the leaf rust population were compared with matching resistance genes in the registered wheat cultivars. The most frequent resistance gene was *Lr37*, while the genes *Lr3a* and *Lr26* were less frequent.

Keywords: leaf rust pathotypes; *Lr* genes; resistance; wheat

In the Czech Republic leaf rust (*Puccinia triticina* Eriks.) is an important pathogen on wheat that often causes considerable yield losses. Breeding for rust resistance and growing resistant cultivars is the most economical way of protection against rust. Knowledge of virulence in the rust population is important for successful resistance breeding. The importance of rust virulence surveys was recently stressed by Park *et al*. (2011). In the Czech Republic virulence in the rust population has been studied since the sixties of the last century, first on the standard differential cultivars (Johnston & Browder 1966) and on an additional differential Salzmünder Bartweizen (*Lr26*). Results of these studies carried out in the years 1966–2001 were summarized by Hanzalová and Bartoš (2014). Later on determination of virulence was based on reactions of leaf rust isolates on a set of Thatcher near-isogenic lines (NILs) possessing *Lr* genes. This research report presents results of the virulence survey in relation to leaf rust resistance genes in the wheat cultivars grown in 2002–2011 and represents a continuation of the 1966–2001 race survey.

MATERIAL AND METHODS

For the rust virulence studies near-isogenic lines possessing *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr9*, *Lr10*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr19*, *Lr21*, *Lr23*, *Lr24*, *Lr26* and *Lr28* in cv. Thatcher background were used. NIL *Lr10* was not used in 2009–2011 when *Lr13* was used instead. For this reason NILs *Lr10* and *Lr13* were not considered in the final classification of pathotypes. Samples of wheat rust were obtained every year from different cultivars, mostly from the variety trials located across the country and organized by the Central Institute for Supervising and Testing in Agriculture, Czech Republic. Rust was propagated on a susceptible cultivar and single pustules were isolated. After inoculation with water urediospore suspension wheat seedlings grown in pots were covered with closed glass cylinders to keep

high air humidity and incubated for 24 h. Thereafter plants were kept in the greenhouse at temperatures 18–22°C. After 10–14 days infection types were scored according to Stakman *et al*. (1962). Results were published in papers that are available at the authors. Leaf rust resistance genes (*Lr*) in wheat cultivars registered in the Czech Republic were determined in multipathotype tests, later they were determined by molecular markers for *Lr10*, *Lr26*, *Lr34* and *Lr37*. Results of molecular analyses were mostly summarized in papers on the physiologic specialization of leaf rust or in separate papers.

RESULTS AND DISCUSSION

In 2002–2011 only sporadic incidence of virulence was determined on NILs possessing *Lr9* and *Lr19*. On average, relatively low virulence was found on NILs with *Lr2a*, *Lr2b*, *Lr24* and *Lr28*. The highest frequency of virulence was ascertained on NILs possessing *Lr3a*, *Lr10*, *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21*, *Lr23* and *Lr26* (Table 1). Several changes in the virulence frequency in the leaf rust population appeared in the course of the period under investigation. The most significant one was an increase of virulence frequency on *Lr1* from an average of 8% in the years 2002–2004 to 85% in the years 2009–2011. Whereas virulence frequency to *Lr2a*, and *Lr2b* showed an increase until the year 2006 and then a decrease, virulence frequency to *Lr2c* decreased from 78% in 2002–2004 to 22% in 2009–2011. Virulence frequency to *Lr15* had also an increasing trend from 42% in 2002–2004 to 91% in 2009–2011. In the virulence frequency to other *Lr* lines no distinct trend of an increase or decrease in virulence frequency was observed and fluctuation was mostly small.

Occurrence of the same pathotypes in successive years is summarized in Table 2. In the years 2002, 2003, 2004 and 2005 only one identical pathotype was found among the ten most widespread pathotypes. In the years 2003 and 2004 two pathotypes were identical. In the years 2004 and 2005 one pathotype was identical. No identical pathotypes were revealed in 2005 and 2006, 2006 and 2007, nor in 2007 and 2008. In the years 2008 and 2009, 2009 and 2010, as well as in 2010 and 2011 three identical pathotypes were determined.

If the inoculum in a subsequent year originates from the local overwintering rust mycelium (asexual stage), in winter wheat the occurrence of the same pathotypes as in the preceding year can be expected. On the other hand, if the source of the inoculum is from abroad due to a long-distance transfer of spores by wind, then there is a chance for a higher diversity of pathotypes.

Table 1. Frequency of virulent isolates (%) on the NILs possessing *Lr* genes

Lr genes	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	Average % 2002–2011
Lr1	3	10	12	14	12	21	82	96	92	67	41
Lr2a	8	7	21	18	15	25	44	35	5	9	19
Lr2b	14	33	23	28	25	35	22	39	5	7	23
Lr2c	92	83	61	66	70	72	24	41	13	12	53
Lr3a	94	88	90	94	94	92	78	76	89	64	86
Lr9	0	0	0	0	0	0	0	2	3	2	0.7
Lr10	81	77	92	92	95	88	87	–	–	–	(87)
Lr11	83	93	90	91	–	96	90	93	95	93	92
Lr13	–	–	–	–	–	–	–	100	95	100	(98)
Lr15	20	35	72	75	70	96	92	85	97	91	73
Lr17	97	95	89	67	66	84	78	100	97	78	85
Lr19	0	0	0	1	0	0	2	0	0	0	0.3
Lr21	81	88	95	86	91	87	94	100	95	88	91
Lr23	92	95	85	95	86	76	56	87	98	84	85
Lr24	22	17	2.5	0	0	0	6	4	8	9	7
Lr26	67	50	69	74	72	45	56	67	78	60	64
Lr28	14	5	9	12	12	19	30	33	13	7	15
Total No. of isolates	35	60	53	65	72	46	50	46	64	54	545

Table 2. Number and virulence of identical pathotypes of the ten predominant pathotypes in the successive years

Years	Number	Virulence
2002/03	1	*Lr2c, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23*
2003/04	2	*Lr2c, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23* *Lr2c, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23, Lr26*
2004/05	1	*Lr2c, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23*
2005/06	0	–
2006/07	0	–
2007/08	0	–
2008/09	3	*Lr1, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr26* *Lr1, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23, Lr26, Lr28* *Lr1, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23, Lr26, Lr28*
2009/10	3	*Lr1, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23, Lr26* *Lr1, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23, Lr26, Lr28* *Lr1, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23*
2010/11	3	*Lr1, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23, Lr26* *Lr1, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23* *Lr1, Lr2c, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23, Lr26*

Pathotypes of the ten predominant races are summarized in Table 3. The highest number of genes for virulence was found in the race predominant in 2004. In all the ten predominant pathotypes virulence was found on *Lr3a*, *Lr11*, *Lr15*, *Lr17* and *Lr21.* Differences between the ten predominant pathotypes were based on virulence/avirulence on *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr23*, *Lr26* and *Lr28*. None of the predominant pathotypes was virulent on *Lr9*, *Lr19*, and *Lr24*; only one pathotype was virulent on *Lr28*. Virulence on *Lr24* and *Lr28* was rare also in less frequent pathotypes not recorded in this report whereas virulence on *Lr3a* was very frequent. Among the ten most widespread pathotypes only two were avirulent on *Lr26*, however among less frequently appearing pathotypes avirulence on *Lr26* was found more often. In sporadically appearing pathotypes avirulence on *Lr11*, *Lr13*, *Lr15*, *Lr17*, *Lr21* and *Lr23* was revealed, however it was rare.

Old standard differentials (Johnston & Browder 1966) used in our earlier race surveys contained *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a* and *Lr11* and some additional leaf rust resistance genes. If we classify the ten most widespread pathotypes according to virulence/avirulence on the above-mentioned *Lr* genes, we can approximately designate the pathotypes by numbers according to Johnston and Browder

Table 3. Ten predominant pathotypes (2002–2011)

No.	Virulence on *Lr* genes	Year of incidence	Race number*
1	*Lr2c, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23*	2002	61
2	*Lr2b, Lr2c, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23, Lr26*	2003, 2007	12
3	*Lr2a, Lr2b, Lr2c, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23, Lr26*	2004	17
4	*Lr2c, Lr3a, Lr11, Lr17, Lr21, Lr23, Lr26*	2003, 2004, 2007	61
5	*Lr2c, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23, Lr26*	2003, 2004, 2007	61
6	*Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23, Lr26*	2004, 2007	2
7	*Lr1, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr26*	2008	5
8	*Lr1, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23, Lr26*	2008, 2009, 2010, 2011	5
9	*Lr1, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23, Lr26, Lr28*	2008, 2009, 2010, 2011	5
10	*Lr1, Lr3a, Lr11, Lr15, Lr17, Lr21, Lr23*	2008, 2010, 2011	5

*Johnnson and Browder (1966)

(1966). In this way we recorded similar races like those described in our previous report (Hanzalová & Bartoš 2014). Pathotype numbers 1, 4 and 5 in Table 3 correspond to race 61 common in previous years. Pathotype numbers 7, 8, 9 and 10 represent race 5 that differs from race 61 only by virulence on *Lr1*. Pathotype number 3 corresponds to race 17 that differs from the earlier common race 77 only by avirulence on *Lr1*. Pathotypes numbers 2 and 6 correspond to races 12 and 2, respectively, and were also identified already earlier.

Kolmer *et al.* (2012) tested 133 leaf rust isolates with 23 single sequence repeat markers for molecular genotypes and on 20 lines of wheat with single leaf rust resistance genes (NILs) for virulence phenotypes. Leaf rust samples originated from Germany, Spain, France, United Kingdom, Hungary, Italy, Romania, Turkey, Ukraine, as well as from the Czech Republic and Slovak Republic. Based on molecular marker analysis he divided the isolates into 8 EU groups. Isolates from the Czech Republic and Slovak Republic were identified in all groups except groups EU3 and EU7. This indicates considerable variability of molecular genotypes. Pathotypes of the 19 isolates from the Czech Republic and Slovak Republic mostly differed from each other, only 3 isolates were doubled. In the tests by Kolmer *et al*. (2012) high frequency of virulence was found on *Lr2c*, which agrees with our results from the first half of the investigation period. High frequency of virulence was also revealed on *Lr1*, like in our tests in the second half of the studied time period. The frequency of virulence on *Lr26* and *Lr10* was also high, it was lower on *Lr28* and very low on *Lr9* (average 0.7 % in the investigation period). Pathotypes similar to those of Czech and Slovak leaf rust isolates were found in different European countries. The virulence phenotypes described by Kolmer *et al*. (2012) were similar to those found previously in Europe and described by Park and Felsenstein (1998). High frequency of virulence on *Lr2c* was also recorded in France (Goyeau *et al*. 2006). A sudden change from avirulence to virulence on *Lr1*, *Lr3a*, *Lr10*, *Lr13*, *Lr14*, *Lr16*, *Lr26* and *Lr37* since 2006 was described in Germany (Serfling *et al*. 2011). Virulence frequency on *Lr1* increased in Russia in the years 2001–2006 in central parts of the country and decreased in the northwestern region (Gultyaeva *et al*. 2000). In our virulence survey virulence on *Lr9* was recorded in the last three years of the investigation period (2009–2011). In the year 2008 virulence on *Lr9* was recorded in Slovakia. It was mentioned only once in the extensive study of leaf rust virulence in Europe by Mesterházy *et al*. (2000) and was not found either in Germany or in Russia by Lind and Gultyaeva (2007) in the years 2001–2003. Virulence on *Lr19* recorded in our tests in 2005 and 2008 was identified in Russia in 1999 (Gultyaeva *et al*. 2000).

Virulence frequency on *Lr24,* relatively high in 2002–2004 in our survey and low later on, was very rare in Germany and it was not found in Russia in the period 2001-2003 (Lind & Gultyaeva 2007).

Changes in virulence do not seem to be affected by resistance genes in the grown cultivars. The only Czech cultivar that possessed *Lr1* was Vlada, registered in 1990. However its hectarage was limited and in 2006

Table 4. Registered cultivars possessing gene *Lr37*

Cultivar	Registered	Multiplication area maximum ha (year)*
Aladin	2010	78 (2012)
Altigo	2011	375 (2011)
Anduril	2006	613 (2008)
Apache	1999	706 (2001)
Bakfis	2008	830 (2009)
Beduin	2011	228 (2012)
Bill	2002	2141 (2003)
Biscay	2005	885 (2006)
Bodyček	2010	1026 (2012)
Caphorn	2004	103 (2004)
Clarus	2003	690 (2004)
Clever	2002	1450 (2003)
Corsaire	1999	1193 (2002)
Dagmar	2012	100 (2012)
Elly	2010	1195 (2012)
Globus	2003	1055 (2006)
Golem	2011	195 (2012)
Graindor	2010	102 (2012)
Ilias	2003	1864 (2005)
Jindra	2010	737 (2011)
Kodex	2008	86 (2008)
Manager	2007	63 (2008)
Matylda	2011	152 (2012)
Mulan	2007	2597 (2009)
Orlando	2008	23 (2008)
Potenzial	2012	1987 (2012)
Rapsodia	2003	1517 (2006)
Rheia	2002	1566 (2006)
RW Nadal	2010	–
Sultan	2008	1209 (2011)
Vanessa	2012	–

*data from the Central Institute for Supervising and Testing in Agriculture, Brno

cv. Vlada was restricted from the registered cultivars. No substantial change was observed in the frequency of virulence to *Lr26* in the studied period though the area of cultivars possessing *Lr26* decreased considerably. In the period 2002–2011 the majority of the grown winter wheat cultivars were susceptible to the wheat leaf rust population of that time. A dominant cultivar in the years 2001–2005 was Sulamit. In 2001–2002 cv. Nela also belonged to cultivars with a large seed multiplication area. In 2005–2007 cv. Ludwig was at the second place whereas in 2006–2009 cv. Akteur dominated in the seed multiplication area. In 2008–2010 cv. Bohemia ranked the second or third in the seed multiplication area. Among these above-mentioned predominant cultivars no *Lr* leaf rust resistance genes were postulated except for *Lr3a* in cv. Bohemia. If we consider other cultivars with smaller seed increase areas in the years 2001–2011, then *Lr3a* was possessed also by cultivar Niagara, gene *Lr34* (APR) by cv. Brea. The most important leaf rust resistance gene in the period 2002–2011 was *Lr37*, mostly expressed only at adult plant stage. It is possessed by registered cultivars listed in Table 4. In the Czech Republic gene *Lr37* lost effectiveness in field conditions around 2010, however some cultivars possessing *Lr37* (e.g. Orlando) remained resistant due to other leaf rust resistance genes.

Our leaf rust virulence survey carried out in the years 2002–2011 confirmed conclusions published earlier. No significant relations between resistance genes in the grown wheat cultivars and virulence genes in the leaf rust population were observed. Similarities in the pathotypes and earlier defined races were ascertained. Pathotypes (virulence phenotypes) recorded in our tests were similar to those described by Kolmer *et al.* (2012) in different European countries, which suggests an interchange of leaf rust inoculum in the European territory.

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