Physiologic Specialization of Wheat Leaf Rust (*Puccinia triticina* Eriks.) in the Czech Republic in 2001–2004

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Abstract: In 2001–2004 virulence of the wheat leaf rust population in the Czech Republic was studied on Thatcher near-isogenic lines with *Lr1, Lr2a, Lr2b, Lr2c, Lr3a, Lr9, Lr10, Lr11, Lr15, Lr17, Lr19, Lr21, Lr23, Lr24, Lr26* and *Lr28*. Samples of leaf rust (180 in total) were obtained from different parts of the Czech Republic. Resistance genes *Lr9* and *Lr19* were effective to all tested isolates like in the previous years. Unlike the previous years virulence on *Lr24* and *Lr28* was observed. Relatively effective were also *Lr1, Lr2a, Lr24, Lr28* and *Lr2b*. Other *Lr* genes were defeated by the majority of the tested samples. Our results transformed to the numbers of physiologic races indicate that race 61SaBa prevailed like in the previous years, followed by races 61, 2, 12SaBa, 2SaBa, 14, 77SaBa, 12, 57, 6, 53, 53SaBa, 77, and 14SaBa. Twenty-one winter wheat cultivars registered in 2001–2004 were tested with 8 leaf rust isolates. Out of them 15 showed resistance at least to one rust isolate. High resistance recorded in field trials for cvs Batis and Ilias, which were susceptible to all tested rust isolates at the seedling stage, demonstrates the importance of field (partial) resistance.

Keywords: *Lr* genes; physiologic races; resistance; winter wheat

Physiologic specialization of wheat leaf rust was first described by Mains and Jackson (1926). They also developed the first set of differential cultivars based on commercial cultivars. Several modifications have been proposed since then, three of the original differential cultivars were dropped and the set composed of 8 cultivars Malakof, Carina, Brevit, Webster, Loros, Mediterranean and Democrat was later used worldwide. Physiological races were numbered (Johnston & Browder 1966). When near-isogenic lines possessing single leaf rust resistance genes were developed, they started to be used as differentials. The term physiologic race was mostly replaced by the term pathotype. The pathotype was defined by enumeration of effective and ineffective resistance genes to the tested rust isolate. To simplify the designation of pathotypes, near-isogenic lines were ranked to fixed triplets which enabled to express virulence/avirulence by code numbers. Recently a set of 15 near-isogenic lines in triplets was proposed by the participants in the international COST 817 Project (Mesterházy *et al.* 2000).

Virulence in the leaf rust populations (physiologic races, pathotypes) is being surveyed in all countries where deliberate breeding for leaf rust resistance as the most economic way of protection against rust has started. In the Czech Republic the physiologic specialization of leaf rust has been studied since the sixties of the last century (Šebesta & Bartoš 1968, 1969; Bartoš & Šebesta 1971). This contribution presents data from the surveys carried out in the years 2001–2004 and results of a greenhouse test for leaf rust resistance of winter wheat cultivars registered in the same years.

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MATERIAL AND METHODS

Samples of wheat leaf rust were obtained mainly from trials of the Central Institute for Supervising and Testing in Agriculture. They were increased on the susceptible cultivars Diana or Nela. Single pustule isolates were obtained from wheat leaves and multiplied on the same cultivars. Inoculation was performed with a water suspension of urediospores on wheat seedlings. Inoculated plants were kept in closed glass cylinders to provide high air humidity for 24–48 h. Infection types were evaluated according to Stakman et al. (1962) 10–14 days after inoculation when plants were kept in a greenhouse at temperatures of 18–22°C and races were numbered according to Johnston and Browder (1966). Virulence in the leaf rust population was determined according to reactions of the studied rust isolates on Thatcher near-isogenic lines agreed as leaf rust differentials by participants in the international COST 817 Action (Mesterházy *et al.* 2000). These lines possess *Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr9, Lr11, Lr15, Lr17, Lr19, Lr21, Lr23, Lr24, Lr26* and *Lr28*. In addition NIL with *Lr10* was also used. In 2001 thirty-two samples from 17 localities, in 2002 thirty-five samples from 11 localities, in 2003 sixty samples from 24 localities and in 2004 fifty-three samples from 15 localities were analyzed. Twenty-one winter wheat cultivars registered in the Czech Republic in 2001–2004 were tested with 8 leaf rust isolates possessing various combinations of virulence using similar methods as described above. Results were compared with the data on resistance presented by the Central Institute for Supervising and Testing in Agriculture of the Czech Republic, in the Survey of Cultivars 2003, 2004, 2005. Scale 1 to 9 (1 – high susceptibility, 9 – high resistance) is used in the trials of the above-mentioned institute.

RESULTS

Results were summarized for individual years and expressed as the percentage of total virulence on individual leaf rust resistance genes. Because the set of the applied 15 near-isogenic lines (see above) contains resistance genes possessed by the original 8 standard differentials Malakof (*Lr1*), Carina (*Lr2b*), Brevit (*Lr2c*), Webster (*Lr2a*), Loros (*Lr2c*), Mediterranean (*Lr3*), Hussar (*Lr11*) and Democrat (*Lr3*) (except gene combinations with *LrB* gene), race numbers could be estimated for the isolates determined on the set of near-isogenic lines. This estimation of races was carried out to demonstrate the continuity between old findings and the situation in the last four years. Suffix SaBa was used to designate virulence on the cv. Salzmünder Bartweizen, i.e. on *Lr26* (Bartoš *et al.* 2001a, b), which is also included in the set of NILs agreed at the COST Action 217.

Near-isogenic lines possessing leaf rust resistance genes *Lr9* and *Lr19* were resistant to all tested isolates. Relatively effective were also resistance genes *Lr1*, *Lr2a*, *Lr24*, *Lr28* and *Lr2b* (average incidence of virulence on them was below 20%). The effectiveness of other *Lr* genes was defeated by the majority of the tested leaf rust samples in 2001–2004. Their ranking according to their effectiveness in descendent order was as follows: *Lr15, Lr26, Lr2c, Lr10, Lr11, Lr21, Lr23, Lr3* and *Lr17*. During the experimental years an increase in virulence was observed on *Lr1, Lr2a, Lr21*, a decrease on *Lr24* and *Lr28* (Table 1). The most widespread pathotypes expressed by code numbers of 5 triplets were 03562, 03762 and 03563.

The majority of the samples conformed with race 61SaBa, followed by races 61, 2, 12SaBa, 2SaBa,

Table 1. Frequency of virulent isolates on the NILs in 2001–2004

Lr genes	Virulent isolates (%)				Average (%)
	2001	2002	2003	2004	
Lr1	6	3	10	12	8
Lr2a	3	8	7	21	10
Lr2b	6	14	33	22,7	19
Lr2c	62	92	83	61	75
Lr3	72	94	88	90	86
Lr9	0	0	0	0	0
Lr10	78	81	77	92	82
Lr11	63	83	93	90	82
Lr15	75	20	35	72	52
Lr17	–	97	95	89	94
Lr19	0	0	0	0	0
Lr21	69	81	88	95	83
Lr23	68	92	95	85	85
Lr24	16	22	17	2,5	14
Lr26	–	67	50	69	62
Lr28	28	14	5	9	14

Table 2. Leaf rust races estimated in 2001–2004*

Year	Number of isolates															
	61	61SaBa	53	53SaBa	77	77SaBa	57	14	14SaBa	2	2SaBa	6	12	12SaBa	?	Total
2001	10	1	–	–	–	–	1	4	–	7	–	1	1	–	7	32
2002	7	13	–	–	–	–	1	1	–	1	1	–	1	2	8	35
2003	11	17	1	–	–	1	1	2	1	4	2	1	3	7	9	60
2004	3	13	1	1	1	7	1	–	–	5	8	–	–	5	8	53
Total	31	44	2	1	1	8	4	7	1	17	11	2	5	14	32	180

*Race determination was based on reactions to *Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr11* and *Lr26*

14, 77SaBa, 12, 57, 6, 53, 53SaBa, 77, 14SaBa. Some combinations of virulence that did not fit to the reactions of the above-mentioned races were left as undetermined (Table 2). Geographic distribution of the analyzed leaf rust samples is shown in Table 3. The results did not reveal any relation between certain virulence combinations and certain geographic area.

Table 4 shows the reactions of winter wheat cultivars registered in 2001–2004. The ranking of

Table 3. Geographic distribution of leaf rust races determined in 2001–2004

District	Number of isolates	Races
Brno	4	61SaBa
Břeclav	6	12SaBa, 61, 2
České Budějovice	2	61
Hodonín	5	61SaBa, 61, 2
Jičín	1	61
Klatovy	2	61SaBa, 12SaBa
Kroměříž	3	61SaBa, 2SaBa, 2, 61
Litoměřice	21	12SaBa, 61, 6, 12, 61SaBa, 2, 14, 14SaBa, 77SaBa
Mělník	1	?
Náchod	1	77SaBa
Nymburk	1	6
Opava	12	61SaBa, 61, 14SaBa, 77SaBa, 2
Pardubice	4	12SaBa, 77
Pelhřimov	1	14
Písek	2	61SaBa
Plzeň-jih	4	61, 57
Praha	8	61SaBa, 61, 2, 2SaBa, 12SaBa
Praha-východ	12	61SaBa, 12SaBa, 2SaBa, 2, 77SaBa, 12, 61
Praha-západ	3	61SaBa
Prostějov	11	61SaBa, 61, 12, 57, 14
Přerov	13	61SaBa, 12SaBa, 61, 14, 57, 12
Svitavy	5	61SaBa, 12SaBa
Třebíč	9	61SaBa, 12SaBa, 61,12, 2SaBa, 2
Uherské Hradiště	1	61
Ústí nad Orlicí	18	61, 61SaBa, 14, 53, 2SaBa, 2, 53SaBa
Znojmo	19	61SaBa, 12SaBa, 77SaBa, 61, 2,
Žďár nad Sázavou	4	61SaBa, 57, 12

Table 4. Reactions of winter wheat cultivars registered in the Czech Republic in 2001–2004 to the leaf rust

Cultivar	Registered	Rust isolate								
		1947	4332	333	9071	9077	9095	CH	F	Field*
Akteur	2004	3	3 –	2 +	3 –	3	–	;	–	6
Alibaba	2003	3	3	3	3	3	3	; 1 +	3	3
Banquet	2001	–	3	; 1	3	3 –	2 –	–	–	3
Batis	2001	3	3	3	3	3	3	3 –	3	8
Bill	2002	; 1 +	; 1	; 1	3 –	0 ;	;1–2 +	0 ;	; 1	7
Caphorn	2004	2	;	;	3	; 1	–	; 1	–	8
Clarus	2003	3 –	3	0 ;	3	3	; 1–2	; 1	2 +	8
Clever	2002	3 –	3	;	3	3	;	2 +	3	8
Cubus	2004	3	3	3	3	3	3	3 –	3	5
Darwin	2004	3	3	3	3	3	3	2	–	6.5
Globus	2003	3	3	; 1	3	3	3 –	3	3	8
Hedvika	2004	3	3	3 –	3	3	3	; 1	3	7
Ilias	2003	3	3 –	3	3	3	3	3 –	3	7.5
Karolinum	2003	0 ;	0 ;	0 ;	;	0 ;	;	;	;	7
Meritto	2003	3	3	3	3	3	3	3	3	5
Mladka	2002	3	3	; 1	3	3	2 +	3	3	5
Rapsodia	2003	0 ;	0 ;	0 ;	3	; 1 +	; 1	0 ;	3 –	8
Rheia	2002	3	3 –	; 1 +	3	3	3	3	3	6
Svitava	2001	3	3	; 1 +	3	3	2 +	2 +	; 1	3.5
Trend	2002	3	3	3 –	3	3	3	3	3	5
Windsor	2001	3	3	3 –	3	3	3	2	3 –	6

*Data published by Central Institute for Supervising and Testing in Agriculture
Disease severity: 1 – high (susceptibility), 9 – low, no symptoms (resistance)

tested cultivars according to the ratio of resistant to susceptible seedling reactions in descendent order was as follows: Karolinum, Bill, Rapsodia, Caphorn, Clarus (all these cultivars were classified in the field trials as resistant by high marks 7 or 8). Cultivars Clever, Svitava, Banquet, Akteur and Mladka, which followed, were classified in the field trials by marks 3–6.5. Cultivars Globus, Hedvika, Rheia, Windsor and Alibaba, which were resistant at least to one rust isolate in the greenhouse, were classified in the field by marks 3–8. Finally, cultivars susceptible to all applied rust isolates in the greenhouse were classified in the field by marks 5–8. A good agreement in the classification of resistance in the greenhouse and in the field was found in cultivars resistant to the highest number of isolates. Discrepancies between greenhouse and field results were in the group of cultivars resistant to a lower number of the tested rust isolates. The most striking difference was in cultivars Batis and Ilias, which were susceptible to all rust isolates at the seedling stage in the greenhouse but highly resistant in the field trials.

DISCUSSION

The most noticeable difference between the 2001–2004 data and data from the preceding analysis of virulence in the Czech leaf rust population (Bartoš *et al.* 2001a, b) was the observed virulence on *Lr24* and *Lr28*. To our best knowledge none of these two genes is present in European commercial wheat cultivars. Gene *Lr24* is derived from rye. It was used in Slovakia for a combination with *Lr19* to assure more durable resistance in the lines developed as sources of leaf rust resistance (Šliková *et al.* 2004). Gene *Lr28* was derived from *Triticum speltoides*. In Germany virulence on *Lr19*, *Lr24* and *Lr28* was found already in 1999 (Gultyaeva *et al.* 2000). On the other hand, the same authors did not find any virulence on *Lr19* and *Lr24* in the leaf rust samples from the European part of Russia. In the results of a COST 817 ring test comprising 10 European countries (France, Czech Republic, Germany, Italy, Spain, Hungary, Poland, Bulgaria, Rumania, Slovakia) summarized by Mesterházy *et al.* (2000) no virulence was recorded on *Lr19*. Virulence on *Lr24* was found in Rumania and Bulgaria and in addition virulence on *Lr28* in Italy and Poland.

The presence of *Lr* genes in the grown cultivars is usually considered as a decisive factor for the distribution of corresponding virulence. This can be demonstrated on virulence to *Lr26*. The spread of this virulence started about fifty years ago and was closely linked with the spread of cultivars possessing the translocation from rye T1BL.1RS. This translocation has rust resistance genes *Lr26, Yr9, Sr31* and powdery mildew resistance gene *Pm8*. Stepwise virulence to all these genes appeared and spread except virulence on *Sr31* that was recorded as late as in recent years (1999) in Africa (CIMMYT Panel 2005). Our results indicate that in spite of a very limited growing area of cultivars with T1BL.1RS at present the occurrence of virulence on *Lr26* still remains high though it is decreasing. Data on the occurrence of virulence on *Lr19* and *Lr24* in areas where no cultivars with these genes are grown indicate that also other factors than only the presence of relevant resistance genes affect the incidence of virulence genes.

Comparison of our results and results obtained in previous race surveys shows that the spectrum of leaf rust races has remained similar. Race 61SaBa and 61 prevailed. Race 61SaBa has been the prevailing race since the late seventies (Bartoš *et al.* 1996). Long-term results suggest that the race spectrum is rather conservative. This may be due to the absence of most genes possessed by the differentials in the widely grown cultivars on the one hand; on the other hand some combinations of genes for virulence (or genes linked with them) may offer a certain selective advantage (higher fitness) to the pathogen.

The majority of the other races found in the period 2001–2004 was also determined in 1999–2000. The race spectrum described in our virulence survey is similar to that in Hungary where also races 61, 12 and 77 are widespread together with race 20 (Manninger 2000). A similar race spectrum has also been determined in Slovakia. Virulence to individual *Lr* genes in our tests did not considerably differ from the virulence reported in Poland where also virulence on *Lr28* was recorded (Mesterházy *et al.* 2000).

From the practical aspect particularly data regarding virulence on resistance genes possessed by registered cultivars are of importance. Virulence on *Lr1, Lr3, Lr10, Lr13, and Lr26* genes possessed by some registered cultivars (Bartoš *et al.* 2000) was widespread except virulence on *Lr1*.

Data on field reactions published by the Central Institute for Supervising and Testing in Agriculture show that several cultivars are relatively resistant in the field. This indicates that they must have other resistance genes than those given above or that they possess field resistance. One of these genes is *Lr37* governing resistance at the adult plant stage. Of the cultivars registered in the years 2001–2004 gene *Lr37* was described in cultivars Bill, Clever, Rapsodia, Clarus and Rheia (Bartoš *et al.* 2004). No other specific resistance genes that could account for the resistance of adult plants have been determined by us, though 15 out of 21 cultivars registered in 2001-2004 possess resistance genes effective at least to one of the tested leaf rust pathotypes (Table 4). Specific resistance may be rather complex. So in the cv. Bill genes *Lr3*, *Lr17*, *Lr23* and *Lru* were recorded in addition to *Lr37* by Shu-Chin Hysing *et al.* (2006). In such cases the postulation of resistance genes according to the reactions to a set of selected isolates is difficult.

Field resistance (often designated as partial resistance) also plays an important role in the resistance of recently registered cultivars. This is evident in the cv. Batis and Ilias, which were susceptible to all tested pathotypes, or in cv. Globus, which was resistant only to one very rarely occurring pathotype. All these three cultivars were highly resistant in the field trials. Resistance expressed only in adult plants in the field is common in European wheat cultivars. Winzeler *et al.* (2000) recorded adult plant resistance in 50 out of 72 tested cultivars and breeding lines. Field resistance is not usually so effective as specific resistance, however it is durable and unspecific.

Race/pathotype surveys are important for epidemiological studies, for the forecast of the breakdown of genes for specific resistance and as a source of isolates for genetic studies of resistance. By means of selected rust isolates differences in the genetic background of resistance in the wheat cultivars can be revealed. Specific genes for resistance can be postulated according to the reactions of the tested cultivars to selected rust isolates. Though due to the limited number of the tested leaf rust isolates our results may not cover the whole variability of leaf rust virulence in the Czech Republic, they indicate that no substantial change in virulence important for the grown cultivars has occurred in the last 4 years.

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