Virulence surveys of wheat leaf rust in the Czech Republic and resistance genes in registered cultivars

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Abstract

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The technical report presents a summary of results obtained in the leaf rust race surveys carried out in Czechoslovakia/Czech Republic in the years 1966–2001. The physiologic races were determined using the standard differentials Malakoff (*Lr1*), Carina (*Lr2b*), Brevit (*Lr2c*), Webster (*Lr2a*), Loros (*Lr2c*), Mediterranean (*Lr3*), Hussar (*Lr11*), Democrat (*Lr3*) and the additional differential Salzmünder Bartweizen (*Lr26*). Races 14, 77, 61, 53 and 2 successively dominated in the given period. They mostly appeared in two variants, avirulent and virulent to *Lr26*. Genes for virulence in the leaf rust races were compared with genes for resistance in the registered wheat cultivars listed in the report. Most frequent were the genes *Lr3* and *Lr26*.

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

In the Czech Republic leaf rust (*Puccinia triticina* Eriks.) on wheat occurs regularly every year. It can cause considerable yield losses on wheat particularly in the eastern part of the country with a warmer climate. Breeding for rust resistance and growing resistant cultivars are among the important components of integrated plant protection. Availability of suitable sources of resistance and knowledge of virulence in the rust population are necessary prerequisites for successful resistance breeding. The importance of surveillance of cereal rust pathotypes (races) was recently underlined by Park *et al*. (2011) and it is one of the most important topics in the Borlaug Global Rust Initiative (MacIntosh & Pretorius 2011). This technical report presents results of the race surveys in relation to leaf rust resistance genes in wheat cultivars grown between1966 and 2001. Its objective is to summarize results of the long-term systematic study and to draw conclusions of local and possibly general validity. Analyses of virulence in the leaf rust population in the years after 2001 will be presented in a separate paper because they were carried out on a set of near isogenic lines with different *Lr* genes.

MATERIAL AND METHODS

In Czechoslovakia and later in the Czech Republic virulence in the rust population has been studied since the 1960's, first on the standard differential cultivars Malakoff (leaf rust resistance gene *Lr1*), Carina (*Lr2b*), Brevit (*Lr2c*), Webster (*Lr2a*), Loros (*Lr2c*), Mediterranean (*Lr3*), Hussar (*Lr11*), Democrat (*Lr3*) and on an additional differential Salzmünder Bartweizen (*Lr26*). Later a set of near isogenic lines (NILs) in a cv. Thatcher background was also used for rust virulence studies. For the race analysis samples of wheat rust were obtained from different cultivars every year, mostly from the variety trials located across the country and organized by the Central Institute for Supervising and Testing in Agriculture, Czech Republic. The rust was propagated on a susceptible cultivar and single pustules were isolated. The rust inoculum obtained was inoculated on wheat seedlings grown in pots that were covered with closed glass cylinders to keep high air humidity and incubated for 24–48 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 17 papers that are available from the authors. Leaf rust resistance genes (*Lr*) in wheat cultivars registered in Czechoslovakia/Czech Republic were determined in multi-pathotype tests, later they were determined by molecular markers for *Lr10*, *Lr26* and *Lr37* (Hanzalová *et al.* 2009). Results were mostly summarized in the papers on physiologic specialization of leaf rust mentioned above.

Leaf rust races. Results of the leaf rust race surveys carried out in the territory of former Czechoslovakia in the years 1966–1993 are summarized in Table 1, in the territory of the Czech Republic in the years 1994–2001 in Table 2. Table 3 presents a summary of reactions of the most important races on standard differentials. Table 4 comprises reactions of four important races on 20 NILs with different *Lr* genes.

In the 1960's race 14 was predominant. Of the genes possessed by standard differentials it was virulent only to *Lr2c* (Brevit, Loros) and *Lr11* (Husar), of the tested NILs also to *Lr16*, *Lr17*, *Lr23* and later to *Lr26*. It was gradually replaced by race 77 virulent to all standard differentials (i.e. *Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr3*, *Lr11*, of the tested NILs also to *Lr10*, *Lr3bg*, *Lr3ka*, *Lr10*, *Lr11*, *Lr15*, *Lr16*, *Lr17*, *Lr21*, *Lr23*, *Lr30* and later to *Lr26*, as well). Virulence to *Lr3* and to *Lr26* was found in Czechoslovakia already before cultivars possessing *Lr3* and/or *Lr26* started to be grown. As early as in the first race survey in 1962–1963 Šebesta and Bartoš (1968) found virulence to *Lr26* (cv. Salzmünder Bartweizen) in race 20 and virulence to *Lr3* in several other races. In the following years virulence to *Lr26* was revealed in race 14, 15, 77 (Šebesta & Bartoš 1969; Bartoš & Šebesta 1971; Bartoš 1976). It was also described by Nover *et al.* (1964). Virulence to *Lr26* occurred in the rust population at least 10 years (probably more) before the first cultivars possessing that gene were registered in Czechoslovakia. The first cultivar that carried the 1BL.1RS translocation (*Lr26*) from Petkus rye, cv. Salzmünder Bartweizen, was tested in official trials in the sixties but was not registered. The first cultivars possessing *Lr26* and grown in Czechoslovakia on a larger area were Kavkaz and Aurora from the Soviet Union, both registered in 1972. Races 77 and 14 were recorded during the entire period 1966–2001.

Race 77 was important also in other European countries, e.g. Hungary (Bócsa 1966), Yugoslavia (Boškovic 1965), Soviet Union (Voronkova 1969) and Rumania (Stewart *et al.* 1967). Nover *et al.* (1964) identified race 77 in rust samples from Germany, Bulgaria, Finland, The Netherlands, Norway as well as from Czechoslovakia. Almost all races identified in the course of the race surveys had two variants: avirulent and virulent to *Lr26.* The avirulent variant was usually found earlier than the virulent one, however later on the variant with virulence to *Lr26* was usually predominant. This trend can be explained by the frequency of the grown cultivars with *Lr26*.

Another important race 61 was identified in 1977 for the first time. At first it was avirulent, later virulent to *Lr26*. The incidence of this race showed an increasing trend until 1991. Race 61 was virulent to *Lr2c*, *Lr3* and *Lr11 (*from the standard differentials) and further to *Lr3bg*, *Lr3ka*, *Lr10*, *Lr11*, Lr16, *Lr17*, *Lr21*, *Lr23* and *Lr30*. After the decrease of race 77 race 61 gained more importance not only in Czechoslovakia but also in Poland (Dwurazna, personal communication 1978), Italy (Pasquini *et al.* 1979), Germany (Unger, personal communication). Kolmer *et al.* (2013) confirmed the migration of leaf rust within

Table1. The most important leaf rust races determined in the years 1966–1993 (in %)

Years	Race										T
	77	14	1	14SaBa	77SaBa	61	61SaBa	53SaBa	2SaBa	other	
1966–1969	34.5	34.5	–	–	–	–	–	–	–	31.0	165
1970–1973	47.6	23.2	6.5	5.3	2.4	–	–	–	–	15.0	168
1974–1976	5.4	2.7	1.3	1.3	87.2	–	–	–	–	2.1	149
1977–1980	6.1	8.0	3.6	–	50.2	22.0	–	–	–	10.1	163
1981–1983	–	9.0	–	1.3	3.9	39.4	41.3	–	–	5.1	155
1984–1986	–	7.9	–	6.0	4.7	16.7	42.9	4.7	–	17.1	317
1987–1990	1.6	3.0	–	3.4	4.6	4.6	42.8	23.4	12.4	4.2	368
1991–1993	2.0	–	–	1.0	0.7	9.6	51.0	4.5	13.8	17.4	290

T – total number of isolates, SaBa – virulence to cv. Salzmünder Bartweizen (*Lr26*)

Table 2. Percentage of leaf rust races determined in the Czech Republic in the years 1994–2001

Year	Race														T
	77	14	1	14SaBa	77SaBa	61	61SaBa	53SaBa	2SaBa	62SaBa	12SaBa	12	2	other	
1994	–	–	–	–	–	3	69	–	–	29	–	–	–	–	35
1995	1	–	–	1	7	–	86	–	–	–	4	1	–	–	91
1996	3	3	1	–	8	3	62	–	17	–	2	–	–	1	87
1997	2	–	–	–	25	2	50	–	18	–	–	–	–	3	44
1998	–	–	–	–	17	7	53	–	–	–	10	–	–	13	30
1999	–	–	–	–	15	3	45	–	27	–	3	–	–	7	33
2000	–	–	–	–	3	3	54	–	3	–	23	–	–	14	35
2001	–	12	–	–	–	–	3	–	–	–	–	3	22	29	32

T – total number of isolates, SaBa – virulence to cv. Salzmünder Bartweizen (*Lr26*)

Europe by detailed virulence and molecular studies of leaf rust isolates from nine European countries.

The last important race appearing in the period 1987–1991 was race 53 virulent to *Lr26*. Race 53 was avirulent to all standard differentials except cv. Hussar (*Lr11*). However, it was no longer found in the years 1994-2001. In that period race 2 particularly with virulence to *Lr26* appeared in several years. It was virulent only to the standard differentials Mediterranean and Democrat (*Lr3*) as well as to cv. Hussar (*Lr11*). Race 62 virulent to *Lr26* that differs from race 2 only by virulence to *Lr2c* was found only in 1994, however at a high frequency (29% of the tested isolates).

Single-step mutations can explain the origin of virulence to *Lr26*. If we decide to consider only reactions on standard differentials we can speculate on the origin of the most important determined races also by one-step mutations as follows: Race 61 could originate from race 14 by acquired virulence to *Lr3*, or by acquired virulence to *Lr2a* from race 62, race 53 from race 1 by acquired virulence to *Lr1*, race 2 by acquired virulence to *Lr3* and race 12 from race 61 by acquired virulence to *Lr2b*. However, if we test different isolates of the same race on NILs with other genes than those possessed by standard differentials, we often find differences in virulence to other *Lr* genes in NILs than only to those in standard differentials. This has to be also considered when data from different countries are compared.

If we analyse the speed of the spread of individual races, we see that it was usually very fast. Rapid spread was typical of race 77. In 1973 race 77 virulent to *Lr26* accounted for only 7.6% of all analysed rust samples, whereas in 1975 it was already 92.9 %. Also the spread of race 61 was very fast. In 1979 the population of race 61 was only 5.7%, in 1980 already 61.1%. Virulence to *Lr26* prevailed in this race in 1982. In 1987 the proportion of race 53 virulent to *Lr26* suddenly increased and represented almost one third of the determined rust samples in 1988. Race 2

Table 3. Reactions of the most important leaf rust races on standard differentials (1991–1993)

Race	*Lr1* Ma	*Lr2b* Ca	*Lr2c* Br	*Lr2a* We	*Lr2c* Lor	*Lr3* Med	*Lr11* Huss	*Lr3* Dem
14	R	R	S	R	S	R	S	R
77	S	S	S	S	S	S	S	S
61	R	R	S	R	S	S	S	S
53	R	R	R	R	R	R	S	R
12	R	S	S	R	S	S	S	S
62	R	R	S	R	R	S	S	S
1	R	R	R	R	R	R	R	R
2	R	R	R	R	R	S	S	S

Ma – Malakoff; Ca – Carina; Br – Brevit; We – Webster; Lor – Loros; Med – Mediterranean; Huss – Hussar; Dem – Democrat; R – resistant, S – susceptible

virulent to *Lr26* exceeded 10% in 1987 and reached its peak of 33% in 1990.

If we compare resistance genes in the grown wheat cultivars and genes for virulence in the determined races, we reveal many so called unnecessary genes for virulence. The presence of many "unnecessary" virulence genes in the rust populations suggests that these genes had no negative effect on the pathogen fitness. MacKey (1992) explained the presence of "unnecessary" genes for virulence by their pleiotropic functions. It seems that except for a few necessary genes for virulence, particularly to *Lr26* and *Lr3*, genes for the pathogen fitness played a more important role in the race spectrum in Czechoslovakia.

Resistance genes (*Lr*) in the cultivars registered in the Czech Republic. In the Czech Republic spring wheat plays a minor role. For this reason only winter wheat cultivars are considered in this report.

In the sixties, cultivars from the Soviet Union possessing *Lr3* were grown on a large area. In the period 1966–1971 out of the 9 registered cultivars 4 cultivars (year of registration is in brackets), namely Mironovskaya 808 (1966), Bezostaya 1 (1966), Belocerkevskaya 198 (1967) and Yubileynaya 50 (1971), possessed *Lr3*. Cultivars possessing *Lr3* were resistant to leaf rust just for a short time. Race 14 avirulent to *Lr3* was only predominant in the rust population until 1968.

Another important gene for leaf rust resistance in the seventies was *Lr26*, the so called rye resistance. It appeared in Czechoslovakia in 1972 with the registration of cvs Kavkaz and Aurora, the latter with *Lr3* in addition to *Lr26*. However, it lost effectiveness in the same decade. Cultivars possessing *Lr26* and/or *Lr3* were also important in the following decades, and were represented also by domestic cultivars, e.g. Solaris (1976 – *Lr26*), Istra (1979 – *Lr26*, *Lr3*) and Juna (1979 – *Lr3*). Also several foreign cultivars were registered in the seventies, e.g. cv. Grana (1975 – *Lr3*) from Poland, Sava (1976 – *Lr3*) from Yugoslavia, Ilyichovka (1975 – *Lr3*), Mironovskaya uluchshennaya (1979 – *Lr3*) both from Ukraine. In the eighties cultivars with *Lr26* and/or *Lr3* predominated: Amika (1980 – *Lr26*, *Lr3*), Odra (1981 – *Lr3*), Iris (1983 – *Lr26*), Sabina (1983 – *Lr26*), Danubia (1984 – *Lr26*), Mara (1984 – *Lr3*), Viginta (1984 – *Lr3*), Agra (1985 – *Lr26*, *Lr3*), Hana (1985 – *Lr3*), Roxana (1985 – *Lr26*), Selekta (1985 – *Lr26*), Branka (1988 – *Lr26*, *Lr3*), Sparta (1988 – *Lr26*, *Lr3*), Mironovskaya korotkosteblevaya (from Ukraine 1984 – *Lr26*). A similar trend continued also in the nineties when the following cultivars possessing leaf rust resistance genes *Lr3* and/or *Lr26* were registered: Sofia (1990 – *Lr26*, *Lr3*), Lívia (1991 – Lr26), Vega (1992 – *Lr3*), Senta (1991 – *Lr26*, *Lr3*), Samanta (1993 – Lr3), Sida (1993 – *Lr26*), Mona (1994 – *Lr26*, *Lr3*), Ilona (1994 – *Lr26*, *Lr3*), Trane (1994 – *Lr26*), Astella (1995 – *Lr3*), Asta (1994 – *Lr3*), Athlet (1996 – *Lr26*), Bruneta (1996 – *Lr3*), Niagara (1999 – *Lr3*), Rialto (1999 – *Lr26*), Saskia (1996 – *Lr3*), Sida (1993 – *Lr26*).

In the years 2000–2001 no cultivar carrying *Lr26* was registered. In 2001 two cultivars carrying *Lr3* were registered, namely Banquet and Svitava. Some of the cultivars with determined genes possessed undetermined genes in addition.

Besides genes *Lr26* and *Lr3,* the cultivars Alka (1995) and Siria (1994) carry *Lr10*, cv. Vlada (1990) *Lr1*. These genes were overcome by race 77. Unlike gene *Lr10*, which is ineffective against the most important races, gene *Lr1* was effective except against race 77.

Leaf rust resistance at the seedling stage was observed but not determined in cvs Košútka (1981),

Table 4. Reactions of selected leaf rust races on NILs with different *Lr* genes (1994–1995)

NILs	Race			
	77	61	14	12
Lr1	4	;	;	;
Lr2a	4	;	;1	;1
Lr2b	4	;1	2+	3
Lr2c	4	3	4	4
Lr3	4	4	;	4
Lr3bg	4	3	;	4
Lr3ka	4	4	;	3
Lr9	;	;	;	;
Lr10	4	4	2+	4
Lr11	4	3	4	4
Lr15	4	;	;	;
Lr16	4	4	4	4
Lr17	4	4	4	4
Lr19	;	;	;	;
Lr21	3	4	3	4
Lr23	4	3	4	4
Lr24	;	;1	;1	2
Lr26	;/4	;/3	;/4	;/4
Lr28	;	;	;	;
Lr30	4	4	2+	4

; 1, 2, 2+ – avirulence; 3, 4 – virulence

Blava (1992), Torysa (1992), Rexia (1994), Samara (1995), Complet (2000), Drifter (2000), Samara (1995), Semper (1999), Sepstra (1999), Tower (1998) and Windsor (2001). Many of the cultivars with undetermined resistance genes were of foreign origin. They started to play an important role in the Czech Republic from the beginning of the 21st century. The resistance gene *Lr37* effective mainly at the adult plant stage became the most important leaf rust resistance gene. Gene *Lr37* was identified in cvs Apache and Corsaire (1999) and in many cultivars registered after 2001. In the Czech Republic it lost effectiveness around 2010. Also other genes were identified by Pathan and Park (2006) in some cultivars registered in Czechoslovakia/Czech Republic: *Lr13* (in 8 cultivars), *Lr3ka*, *Lr14a*, *Lr17b* (in single cultivars). In the period of the race survey (1966–2001) cultivars with leaf rust resistance genes counted mostly only for a smaller part of the grown cultivars, the majority being represented by cultivars susceptible to leaf rust at the seedling stage and mostly also at the adult plant stage.

Rapid spread of virulence to *Lr3* and *Lr26* and in consequence the loss of their effectiveness evoke the question why genes *Lr3* and *Lr26* have appeared in the grown cultivars for the entire period of the race survey. The presence of gene *Lr26* can be explained by a positive effect of the translocation 1BL.1RS on the yield. A negative effect on the baking quality explains why, with increasing demands for this trait, cultivars with the rye translocation gradually made way to high quality cultivars. As far as gene *Lr3* is concerned, we speculate that this gene has a positive effect on agronomically important trait(s), e.g. quality or is closely linked with such genes. Further research is needed.

CONCLUSIONS

If we analyse the results, we have to take into account that in some years only a small number of rust isolates was analysed, that the applied standard differentials and set of NILs comprised only a small part of leaf rust resistance genes identified until now and that also data on resistance genes in the tested cultivars were limited. Nevertheless, the tests carried out over the long period suggest the following conclusions:

– The presence of the genes for virulence in the leaf rust population was only partially influenced by resistance genes in the grown cultivars. In most years, cultivars without genes for resistance were predominant among the grown cultivars.
– More different genes for virulence were recorded in the rust population than only those corresponding to the resistance genes in the grown cultivars. A negative effect of "unnecessary" genes on the fitness of the pathogen was not observed. They probably have pleiotropic effects on other traits important for the pathogen.
– Changes in the determined predominant races were relatively fast. Airborne inoculum from southern and south-eastern Europe may have been responsible for them. In the years of the race surveys similar leaf rust races like in Czechoslovakia and later in the Czech Republic were described in many countries particularly in Eastern Europe.
– The most widespread resistance genes in the grown cultivars (*Lr3*, *Lr26*) were effective only for a relatively short time. Virulence to these genes in the rust population was found long before cultivars possessing them started to be grown. Resistance based on *Lr3* and/or *Lr26* in the grown cultivars obviously contributed to the rapid spread of the corresponding virulence.
– Resistance genes *Lr3* and *Lr26* have appeared in registered cultivars until now even though both resistance genes have been ineffective for a long time. As the reason for it a positive effect of *Lr3* and *Lr26* or closely linked genes on agronomic traits required in the wheat cultivars was postulated.

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