Leaf Rust Resistance Genes in Japanese Wheat cultivars

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Leaf rust, caused by *Puccinia triticina* E., is an important disease of wheat in Japan. Thirty Japanese wheat (*Triticum aestivum* L.) cultivars, 7 Japanese supplementary differentials and a set of near-isogenic lines carrying named *Lr*-genes, were evaluated for resistance at the seedling growth stage with an array of Mexican *Puccinia triticina* races. The variation in seedling infection type of the cultivars was compared with that of the differentials, and genes conferring a low infection type were postulated. In total, nine named genes, viz. *Lr1* (2 cultivars), *Lr3* (3), *Lr9* (4), *Lr10* (5), *Lr17* (1), *Lr19* (1), *Lr23* (8), *Lr27* + *31* (3) could be identified/postulated in Japanese cultivars and supplementary differentials.

Key Words: *Triticum aestivum* L., *Puccinia triticina*, Leaf rust, *Lr*.

Introduction

Leaf rust caused by *Puccinia triticina* E. is an important disease of wheat in Japan, especially Hokkaido and Tohoku area. It leads to substantial yield losses. Genetic resistance is the most economical and environmentally safe measure to reduce crop losses. Until recently, more than 40 leaf rust resistance (*Lr*) genes have been identified from different sources and designated (McIntosh *et al.* 1998). Resistance is broadly categorized into two groups: race-specific type and race non-specific type. Some of them, such as *Lr12*, *Lr22a*, *Lr22b*, are adult plant resistance genes that are largely effective only in adult plants. *Lr34* and *Lr46* show slow rusting type of resistance where disease progresses at a retarded rate, resulting in intermediate to low disease levels against all pathotypes of a pathogen (Singh and Rajaram 1992, Singh *et al.* 1998). Little is known about the leaf rust genes present in Japanese wheat cultivars even in the Japanese supplementary differentials (Yamada *et al.* 1960) and the parental lines bred for leaf rust resistance (Yamaguchi 1997).

The ability to diversify the genetic base for resistance depends on available knowledge of resistance genes present in the germplasm used in a breeding program. Named race-

specific genes for resistance to rust in wheat can be postulated if the pathogen possesses diverse combinations of avirulence and virulence genes. This method, which is based on the gene-for-gene relationship, has been described and used by several researchers (Browder and Eversmeyer 1980, Knott and Johnson 1983, Singh 1993, Singh and Gupta 1991, Singh and Rajaram 1991, Singh *et al.* 1999, Statler 1984). Postulations can be further confirmed, if necessary, by genetic analyses.

The objective of the present study was to determine the previously described genes that confer resistance at the seedling growth stage in Japanese wheat cultivars.

Materials and Methods

A set of differential lines, mostly near-isogenic situation for known single genes in a 'Thatcher' (CI 10003) background, 30 Japanese wheat cultivars and 7 Japanese additional differentials (Yamada *et al.* 1960), included in the study are listed in Tables 1 and 2, respectively. Seeds of the differentials, originally provided by the Cereal Disease Laboratory, St. Paul, Minnesota, USA, and the Plant Breeding Institute of the University of Sydney, Australia, are maintained at CIMMYT.

The nomenclature system for the 11 races of *P. triticina* (Table 1) used in the study follows the system of Long and Kolmer (Long and Kolmer 1989) with two additional supplementary sets (Singh 1991). Vacuum-dried (Roelfs *et al.* 1992) urediniospores of these races are stored at CIMMYT. Fresh inoculum was obtained by multiplying urediniospores on the susceptible cultivar Morocco.

Fully expanded primary leaves of 9-day-old seedlings of cultivars and testers were inoculated by spraying urediniospores suspended in the light-weight mineral oil Soltrol 170 (Phillips 66 Company, Oklahoma) at a concentration of 2-3 mg/ml. Different sets of seedlings were used for inoculation with each of the 11 races, and six to eight seedlings, planted in a clump, were used in each test. Inoculated plants were placed in a dark dew chamber overnight at 18 to 20°C, dried for an additional 4 hr under light, and transferred to a greenhouse maintained at 18-23°C. Infection types were recorded after 10 days using a 0-4 scale for leaf rust similar to that described by Stakman *et al.* (1962) for stem rust as following: 0 = no uredinia or other macroscopic signs of infection; - = no uredinia, but hypersensitive necrotic or chlorotic flecks of varying size present; 1 = small uredinia surrounded by necrosis; 2 = small to medium

Communicated by T. Yamada

Received June 19, 2000. Accepted February 13, 2001.

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uredinia surrounded by green islands; X = random distribution of variable-sized uredinia on single leaf with a pure culture; 3 and 4 (= H) = medium to large sized uredinia without chlorosis or necrosis; + = uredinia somewhat larger than normal for the infection type; - = uredinia somewhat smaller than normal for the infection type; c = more chlorosis than normal for the infection type. More than one designation represents a range of infection types. Tests were repeated twice during different months. Infection type data from only one test are reported because of the absence of significant variation in the infection type in the two tests.

The presence of leaf rust resistance genes (*Lr* genes) in seedlings of the cultivars was postulated by comparing the low and high infection types displayed by the cultivars with

the infection type of known *Lr* genes in the differentials (Singh and Rajaram 1991).

Results and Discussion

Variation in infection types (ITs) of the known *Lr*-gene-carrying tester lines, inoculated with 11 *P. triticulturae* races (Table 1), indicated possibility of the identification of 18 resistance genes (*Lr1*, *2a*, *2b*, *2c*, *3*, *3bg*, *10*, *11*, *13*, *15*, *16*, *17*, *19*, *23*, *24*, *26*, *27* + *31*, and *28*). Resistance genes *Lr3ka*, *9*, *21*, *25*, *29*, *30*, *32*, *33* and *35* displayed low or intermediate ITs with all races. Absence of an *Lr* gene is indicated in a cultivar whenever a high IT is observed with any of the races used in the tests. Postulation of genes *Lr12*,

Table 1. Seedling infection type responses of differentials with known *Lr* genes when inoculated with 11 Mexican races of *Puccinia triticulturae*

No.	Differential	<i>Lr</i> gene	<i>P. triticulturae</i> race										
			CBJ QB	CBJ QL	CBJ QQ	CCJ SP	LCJ BN	NCJ BN	TBD TM	TCB TD	MFB SP	MCJ QM	MCJ ¹⁾ SP
1	Thatcher	22b	3 +	3	3	3	3	3 +	3 +	3 +	3 +	3 +	3
2	RL6003	1	0;	0;	0;	0;	3 +	3 +	3 +	3 +	3 +	3 +	3
3	RL6016	2a	;	0;	0;	0;	;	1-	3 +	3 +	0;	0;	0;
4	RL6019	2b	1-	;	1-	0;	;	1 +	3 +	3 +	;	0;	0;
5	RL6047	2c	1	12-	1-	;	X	3	3 +	3 +	1-	0;	;
6	RL6002	3	3 +	23-	3C	23C	;	;	3 +	3 +	3 +	3	3-3
7	RL6007	3ka	12	1-	;	1-	12	1	12	12	12	12-	1
8	RL6042	3bg	3 +	3	3	12	;	;	3 +	3 +	3	3	12
9	RL6010	9	0;	;	0;	0;	0;	0;	0;	0;	0;	0;	0;
10	RL6004	10	;	3 +	3	3	3	3	3 +	1-	3	3	3
11	Hussar	11	3	3 +	3 +	3 +	3	3 +	3C3	1 + 3C	3C3	3	3 +
12	RL6011	12	3	3	3	3	3C	3	3 +	X +	3 +	3	3 +
13	Manitou	13	3 +	3	3	3	X	X-	3 +	3 +	3 +	3	3
14	RL6013	14a	3	3 +	3 +	3	3 +	3 +	3 +	3 +	3 +	3	3
15	RL6006	14b	3 +	3 +	3 +	3	3 +	3 +	3 +	3 +	3 +	3 +	3
16	RL6052	15	0;	;	;	3	1	1-	3 +	3 +	3 +	;	3 +
17	RL6005	16	1 +	11 +	1-	1 +	1 +	1 +	12	1 +	1	1	1 +
18	RL6008	17	3 +	X + 3	3	3 +	3 +	3	3 +	1-	;	3	3 +
19	RL6009	18	3 +	3	2 + 3	3	3	22 +	3 +	3 +	3 +	3 +	3 +
20	RL6040	19	0;	0;	3	0;	0;	0;	0;	;	;	0;	0;
21	Thew	20	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +
22	RL6043	21	12	12	12	23C	12	1-	12	12-	12	12	12
23	RL6044	22a	3 +	3	3	3	3 +	3	3 +	3 +	3 +	3	3 +
24	RL6012	23	1-	1	1	3	3 +	3 +	22 +	3 +	3 +	1	3 +
25	RL6064	24	1-	12	;	;	;	12	12	12	3 +	;	;
26	Transec	25	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;	0;
27	RL6078	26	11 +	1-	0;	3	3C3	3	1-	3 +	3C3	3 +	3
28	Gatcher	10, 27 + 31	;	12-	12	3	12	12	3 +	;	3 +	3 +	3C3
29	CS2D-2M	28	0;	0;	0;	0;	3 +	3	3 +	3	3	0;	0
30	RL6080	29	1	1	;	;	1	1	1	1	1	1	;
31	RL6049	30	12	12	23-	12	12	23-	3C	12	12	1	1-
32	RL5497	32	3C	22 +	12	12	3	3	3	12	12	3C3	12
33	RL6057	33	3 +	3	3	23C	3	3	3	23	2 + 3C	3-	12
34	RL6058	34	3	3	3	3	3 +	3	3	3	3	3	3 +
35	RL5711	35	1 + 3C	23C	1 + 3C	3C3	3C	3C3	3C3	23C	1 +	1 +	1 + 3C
36	E84018	36	1	2 + 3	1 +	1 + 2	1	1 +	1 +	1	;	1	1
37	RL6081	37	3C3	3 +	3C	3 +	3C3	2 + 3	3C3	3C3	2 + 3	1 + 3C	3 +

¹⁾ Prt code (Singh 1991).

14a, 14b, 18, 20, 22a, 22b and 34 was not possible with these 11 races because high ITs were recorded with each race. Gene *Lr18* can be detected with Mexican races only if tested at temperatures below 18°C (Singh 1991). Similarly, *Lr34* can give low ITs at low temperatures combined with low light intensities. Genes *Lr12*, 22a, 22b and 34 are known to be effective only in adult plants (McIntosh *et al.* 1998).

Infection types displayed by the Japanese cultivars,

parental lines and supplementary differentials, inoculated with the 11 *P. triticina* races, are given in Table 2. Sixteen of the 37 Japanese wheat cultivars displayed high infection types with all eleven races of *P. triticina*. Therefore, we were unable to detect any resistance genes in these cultivars. The presence of *Lr1* could be indicated in Norin 31 because this cultivar displayed a low infection type 0; or ; with the four avirulent races, viz. CBJ/QB, CBJ/QL, CBJ/QQ and CCJ/SP (Prt code: Singh 1991), and high ITs with the other

Table 2. Seedling infection types displayed by Japanese wheat cultivars and additional leaf rust differentials when tested with 11 Mexican races of *Puccinia triticina*

No.	Cultivar	<i>P. triticina</i> race and infection type											Postulated <i>Lr</i> genes
		CBJ QB	CBJ QL	CBJ QQ	CCJ SP	LCJ BN	NCJ BN	TBD TM	TCB TD	MFB SP	MCJ QM	MCJ ¹⁾ SP	
<i>Japanese cultivars:</i>													
1	Chihokukomugi	X +	X	X	3 +	X	X	3 +	X	3	3 +	3 +	<i>Lr27 + 31</i>
2	Taisetsukomugi	;13C	2	;1	3 +	1 + 2	X	X	;1	;1–	;12	3 +	<i>Lr17, 23, 27 + 31</i>
3	Takunekomugi	;1	;1	;1	3 +	X	X	2 + 3c	X +	2 + 3	;1	3 +	<i>Lr23, 27 + 31</i>
4	Horoshirikomugi	3 +	3 +	3	3 +	3 +	3 +	3 +	4	3 +	3 +	3 +	^{–2)}
5	Haruhikari	3 +	3C3	3	3 +	3C3	3	3 +	3 +	3	3	3 +	–
6	Haruyutaka	3 +	3 +	3	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	–
7	Nanbukomugi	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	–
8	Kitakamikomugi	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	–
9	Akitakko	3 +	3 +	3 +	3	3 +	3 +	3 +	3 +	3 +	3 +	3 +	–
10	Koyukikomugi	;1–	;1–	;1–	3 +	3 +	3 +	2 + 3C	3 +	3 +	;	3 +	<i>Lr23</i>
11	Shiranekomugi	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	–
12	Bandouwase	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3	3 +	3 +	–
13	Fukuhokomugi	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	–
14	Shirasagikomugi	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3	3 +	3 +	–
15	Asakazekomugi	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3	3 +	3 +	–
16	Nishikazekomugi	3	3 + 4	3 +	3 +	3 +	3 +	3 +	4	3 +	3 +	3 +	–
17	Norin 61	3	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	–
18	Shiroganekomugi	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3	3 +	3 +	–
<i>Japanese parental lines for leaf rust resistance:</i>													
19	Wheat Parental Line No. 1	0;	0;	0;	0;	0;	0;	;	0;	0;	0;	0;	<i>Lr9?</i>
20	Wheat Parental Line No. 2	;	;1	;12	3 +	3 +	3 +	;22 +	;	3 +	;1–	3 +	<i>Lr10, 23</i>
21	Wheat Parental Line No. 3	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	–
22	Wheat Parental Line No. 6	0;	0;	0;	0;	0;	0;	;	;	;	0;	0;	<i>Lr19, +³⁾</i>
23	Sabiho 20	;	3C3	3 +	3	2 + 3	3 +	3	;	;11 +	23C	3 +	<i>Lr10, +</i>
24	Sabikei 23	3 +	3	23C	3	2 + 3	3C	12	12	23C	3	3 +	+
25	Sabikei 24	;	;1–	;	3	3 +	3 +	;12	;	3	;1–	3	<i>Lr10, 23</i>
26	Sabikei 27	0;	0;	0;	0;	0;	0;	;	0;	0;	0;	0;	<i>Lr9?</i>
27	Sabikei 30	;	12	12	3	3 +	3 +	3C3	;	3 +	12	3 +	<i>Lr10, 23</i>
28	Sabikei 31	0;	;	0;	0;	0;	0;	0;	;	0;	0;	0;	<i>Lr9?</i>
29	Sabikei 36	0;	;	0;	0;	0;	0;	0;	0;	0;	0;	0;	<i>Lr9?</i>
30	Sabikei 52	;1–	3 +	3 +	3	3	3 +	3 +	;1–	3	3 +	3 +	<i>Lr10</i>
<i>Japanese additional differentials:</i>													
31	Norin16	3 +	3 +	3 +	3 +	3 +	3 +	3	3 +	3	3 +	3 +	–
32	Norin 55	1	;	12	3 +	3 +	3 +	2 + 3	3 +	3 +	;1	3 +	<i>Lr23</i>
33	Norin 31	0;	;	0;	0;	3 +	3 +	3 +	3 +	3 +	3 +	3 +	<i>Lr1</i>
34	Aobakomugi	3	3 +	3 +	3 +	;	;	3 +	3 +	3 +	3 +	3 +	<i>Lr3</i>
35	Norin 62	3 +	23C	3 +	23C	;	;	3 +	3 +	3 +	3 +	23	<i>Lr3</i>
36	Akasabishirazu 1	0;	0;	0;	0;	;	;	3	3 +	3 +	1 +	;1	<i>Lr1, 3, +</i>
37	Eclipse	1	;	X–	3 +	3 +	3 +	3 +	3 +	3 +	12	3 +	<i>Lr23</i>

¹⁾ Prt code (Singh 1991)

²⁾ Not detected

³⁾ Additional genes are present.

Table 3. Leaf rust resistance genes identified in Japanese wheat

Lr gene(s)	Cultivar(s)
Lr1	Norin 31
Lr3	Aobakomugi, Norin 62
Lr9	Wheat Parental Line No. 1, Sabikei 27, Sabikei 31, Sabikei 36
Lr10	Sabikei 52, Sabiho 20
Lr19, + ¹⁾	Wheat Parental Line No. 6
Lr23	Koyukikomugi, Norin 55, Eclipse
Lr27 + 31	Chihokukomugi
Lr1, 3	Akasabishirazu 1
Lr10, 23	Wheat Parental Line No. 2, Sabikei 24, Sabikei 30
Lr23, 27 + 31	Takunekomugi
Lr17, 23, 27 + 31	Taisetsukomugi

¹⁾ additional genes are present.

seven virulent races. Gene *Lr3* could be identified in Aobakomugi and Norin 62 because of their low ITs with races LCJ/BN and NCJ/BN intermediate or high ITs with other races. In a similar manner the presence of other known genes, either singly or in combination could also be indicated (Tables 2, 3). In total, the genes that could be present in Japanese wheat are *Lr1* (2 cultivars), *Lr3* (3), *Lr10* (5), *Lr17* (1), *Lr23* (8), *Lr27 + 31* (3 cultivars) (Table 3). *Lr27* and *Lr31* are the complementary genes that are not expressed in the absence of another complementary factor (Singh and McIntosh 1984).

Five cultivars, Wheat Parental Line No. 1, Wheat Parental Line No. 6, Sabikei 27, Sabikei 31, Sabikei 36, displayed low ITs 0; or ; with all races (Tables 2). 'Agrus', known to carry the *Lr19* gene (McIntosh *et al.* 1998), in the pedigree and yellow endosperm of Wheat Parental Line No. 6 indicated the presence of *Lr19* in this wheat (Yamaguchi *et al.* 1993). However, Wheat Parental Line No. 6 must carry an additional gene that gave resistance to the *Lr19*-virulent race CBJ/QQ. The pedigree of Sabikei 36 indicated the presence of *Lr9* gene from 'Transfer' (McIntosh *et al.* 1998). Virulence for *Lr9* is absent in Mexican population of *P. tritici*. Unpublished data of R. A. McIntosh, University of Sydney, Australia, also indicate the presence of *Lr9* in some Sabikei wheat lines. Based on the low seedling infection type, we therefore, postulate the presence of *Lr9* in the Wheat Parental Line No. 1, Sabikei 27, Sabikei 31 and Sabikei 36.

A similar study using leaf rust races collected from various parts of Japan during 1952 to 1958 was done to establish the Japanese nomenclature system of leaf rust races (Yamada *et al.* 1960). In that study, the Japanese wheat varieties were classified into 7 groups by their reactions to the Japanese leaf rust races. Seven Japanese additional differentials were selected from these 7 groups. Nanbukomugi was classified into the same group as Norin 55 using the Japanese races. When classified using the Mexican races, Nanbukomugi showed an infection type different from Norin 55, but the same as Norin 16. It is supposed that Nanbukomugi and Norin 55 have resistance genes, other than *Lr23*, which categorized them into differential groups

different from Norin 16. Eclipse showed the same infection type as Norin 55 when classified using Mexican races, which implied the presence of *Lr23*. Eclipse is an important differential used to classify the biotypes of the Japanese leaf rust races (Yamada *et al.* 1960) and showed an infection types different from that of Norin 55. Thus, Eclipse may also have resistance genes, which Norin 55 does not have, distinguishing the biotypes of the Japanese races. The Mexican races could not reveal the presence of these genes, which were shown by the Japanese races.

This study showed that some Japanese additional differentials and parental lines have resistance genes, which are different from the previously described ones and designated them as *Lr* genes. Further research should be undertaken to broaden the basis of genetic resistance in Japanese wheat cultivars.

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