Rust Resistance of the French Wheat Cultivar Renan

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Abstract*:* Our field experiments confirmed the leaf rust resistance of cv. Renan in the Czech Republic. Whereas the leaf rust resistance gene *Lr37* possessed by Renan is generally effective as late as at the adult plant stage, we found one leaf rust isolate that caused resistant to moderately resistant reactions on NIL *Lr37* as well as on the cv. Renan already at the seedling stage. This isolate was used in the study of genetics of the leaf rust resistance of cv. Renan in greenhouse experiments. The presence of translocation from *Aegilops ventricosa* carrying the cluster of rust resistance genes *Lr37, Sr38* and *Yr17* was also determined by a PCR molecular marker. All experiments confirmed the presence of *Lr37* gene in cv. Renan. The presence of *Lr14a*, postulated earlier, could not be verified. The resistance of cv. Renan in the field was slightly higher than that of the line Tc/8//VPM1 possessing *Lr37*, which may indicate a more complex genetic base of leaf rust resistance in the cv. Renan. In the progeny of the cross Boka/Renan leaf rust resistance gene *Lr37* behaved as a recessive or partially dominant gene, stem rust resistance gene *Sr38* as a dominant gene.

Keywords: winter wheat; cv. Renan; leaf rust resistance; *Lr37*; *Sr38*

Cultivar Renan is a French winter wheat cultivar selected from a complex cross of Mironovskaya 808/Maris Huntsman//VPM/Moisson/3/Courtot (Martynov *et al.* 2006). In France it was registered in 1989 and has been widely grown since then. Cv. Renan possesses the gene cluster in a translocation from *Aegilops ventricosa* that carries genes for resistance to leaf rust (*Puccinia triticina* Eriks.), yellow rust (*Puccinia striiformis* Westend.) and stem rust (*Puccinia graminis* Pers.), *Lr37, Yr17* and *Sr38,* respectively (Robert *et al.* 1999). The objective of our study was to contribute to the knowledge of disease resistance of cv. Renan as a prospective cultivar for wheat breeding programs.

MATERIAL AND METHODS

Genetic study of the leaf rust resistance of cv. Renan was based on crosses Boka/Renan; one trial was carried out with the cross Renan/Arina. The seed of cv. Renan originated from INRA-Grignon (France), seed of cvs. Boka and Arina from the Gene Bank, Crop Research Institute, Praha-Ruzyně. Cv. Boka is susceptible to leaf rust at the seedling stage and is moderately susceptible at the adult plant stage probably due to the APR gene *Lr13* postulated by Pathan and Park (2006). Near isogenic Thatcher lines NIL *Lr37* (Tc/8//VPM1) and NIL *Lr14a* (Selkirk//6/Thatcher) were developed and studied by Dyck and Samborski (1970). We

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obtained these lines by courtesy of Dr. M. Csösz (Szeged). The presence of gene *Lr37* in the studied crosses was investigated by an infection test with a suitable leaf rust race or by means of a molecular marker.

Infection tests on seedlings were carried out by rubbing the first leaf with a water urediospore suspension by fingers. Then plants were sprayed with water and incubated at high air humidity in closed glass cylinders for 24 hours. After two weeks when inoculated plants were kept in a greenhouse at 20 ± 2°C, infection types (IT) were scored according to Stakman *et al.* (1962), 0 – no visible uredia, ; – hypersensitive flecks, 1 – small uredia with necrosis, 2 – small to medium sized uredia with green islands and surrounded by necrosis or chlorosis, 3 – medium sized uredia with or without chlorosis, 4 – large uredia without chlorosis. In the field under natural infection with leaf rust disease severity (%) and infection types (R, MR, MS, S) of the tested plants/progenies were scored.

To determine the translocation from *Aegilops ventricosa* carrying the cluster of rust resistance genes a PCR (Vlr2) marker according to a modified protocol by Seah *et al.* (2001) was applied. The reaction mixture consisted of 100 ng of genomic DNA, 5 µl 10 × PCR buffer (Promega), 4 µl 25mM $MgCl_2$, 5 µl 2mM dNTPs, 5µl of each 10 pmol/µl primer, 22.5 µl H_20 and 1U Taq polymerase. The PCR amplifications were done in a Techne Flexigene cycler under the following conditions: 2 min denaturation at 94°C followed by 35 cycles of 1 min denaturation at 94°C, 2 min annealing at 61°C and 2 min extension at 72°C followed by final extension 3 min at 72°C. The amplified fragments were separated on 2% agarose gel (Serva) and visualised under UV light after staining with ethidium bromide. A 100bp DNA ladder (Fermentas) was loaded onto the gel together with the amplified fragments.

Greenhouse tests of F_1, F_2 and F_3 were carried out in the Crop Research Institute, Prague-Ruzyně, field tests of F_1, F_2 also in Prague-Ruzyně, tests of F_3 and further generations at Selgen Plant Breeding Station, Úhřetice, under natural leaf rust infection. Leaf rust reaction was tested in parental cultivars, in F_1, F_2 and F_3 generations of the cross Boka/Renan. Leaf rust isolate 333, applied in the tests, was avirulent both to *Lr37* and to *Lr14a*. Isolate CH was avirulent on NIL *Lr37* and had a heterogeneous reaction on NIL Lr*14a* (Goyeau, personal communication). The most resistant plants from F_3 progenies in the field were selected and scored again in F_4 and F_5. Two plants were reselected in each generation. In F_6 seedlings the presence of *Lr37* was tested in the greenhouse. A part of F_2 population from the cross Boka/Renan and Renan/Arina was tested also with the stem rust isolates avirulent to *Sr38* (isolates G 324 and G 927, respectively) and virulent to the other parent of the relevant cross and with leaf rust isolate 333 or isolate CH. Stem rust was inoculated on the first leaf, leaf rust five days later on the second leaf. Infection types were scored 3 weeks after the first inoculation.

Table 1. Segregation for the reaction to *Puccinia triticina* in progenies of the cross Boka/Renan – seedling reaction

Generation	Number of plants/progenies*				χ^2	*P*
	R	S	segregating* (F_3 progenies)	total	1:3 / 1:1:2*	
P – Renan	10	0	–	10	–	–
P – Boka	0	10	–	10	–	–
F_1	0	14	–	14	–	–
F_2 experiment A	34	117	–	151	0.494	0.5–0.2
F_2 experiment B	30	72	–	102	1.058	0.5–0.2
A + B	64	189	–	253	0.012	0.99–0.95
F_3*	13	17	40	70	1.87	0.5–0.2

R – resistant, S – susceptible

Because of limited greenhouse space F_2 generation was tested in two successive experiments (A and B)

Table 2. Segregation of the reaction to *Puccinia triticina* and *Puccinia graminis* in F_2 generation of the crosses Boka/Renan and Renan/Arina (isolate CH* = B950506A, INRA, Grignon)

Cross	Isolate		Number of plants			χ^2	*P*
	P. triticina	*P. graminis*	R	S	Σ		
Boka/Renan	333	–	25	84	109	1:3 0.24	0.8–0.5
	–	G 324	86	24	110	3:1 0.59	0.5–0.2
Renan/Arina	CH*	–	17	36	53	1:3 1.41	0.5–0.2
	–	G 927	35	15	50	3:1 0.67	0.5–0.2

R – resistant, S – susceptible

RESULTS

Greenhouse experiments

Whereas cv. Boka displayed IT 3 and cv. Renan IT ;1 2, F_1 generation of the cross was susceptible to medium susceptible IT 3(3–) indicating recessive resistance (or partial dominance). This was confirmed in F_2 generation by segregation at the ratio 3 IT 3(3–):1 IT ;1 2. Resistant reaction varied from IT ;1 to IT 2 sometimes with large chloroses or necroses. The scoring of F_3 generation also supported the presence of one gene (Table 1).

Greenhouse tests with both leaf and stem rust revealed that *Sr38* was dominant whereas *Lr37* behaved as a recessive gene (Table 2). All plants except one in the cross Boka/Renan that were resistant to leaf rust were also resistant to stem rust. In the cross Renan/Arina no such a case of discrepancy was found. In a few cases only one rust species could be scored because of infection escape.

In the greenhouse tests seedling reactions of cv. Renan were compared with NIL *Lr37* and NIL *Lr14a* (Table 3). Compared with Renan, NIL *Lr37* displayed a slightly higher, less resistant infection type to leaf rust isolate 333. Rust isolates 1947 and 347 were virulent to NIL *Lr37* and Renan but avirulent to NIL *Lr14a*.

Table 3. Reactions of cv. Renan and NILs *Lr37* and *Lr14a* to three leaf rust isolates

Cultivar/line	Isolate/IT		
	333	1947	347
Renan	;1	3	3
NIL *Lr37*	;1+	3	3
NIL *Lr14a*	;1	2	2

Infection types (IT) after Stakman *et al.* (1962) – ; 1, 1, 1+ 2 – resistant, 3 – susceptible

Table 4. Reactions of F_3 plants of the cross Boka/Renan in a greenhouse infection test with stem rust (G324) and leaf rust (333) isolates and results of analysis by the molecular marker Vlr2 according to Seah *et al.* (2001)

Plant number	Reaction		
	stem rust	leaf rust	marker
1	R	S	+
2	R	S	+
3	R	S	+
4	R	R	+
5	R	R	+
6	R	R	+
7	R	R	+
8	S	S	–
9	S	S	–
10	S	S	–
11	MR	S	+
12	MR	S	+
13	MR	R	+
14	MR	R	+
15	MR	R	+
16 NIL *Lr37*	R	R	+
17 Boka	S	S	–
18 Renan	R	R	+

R – resistant, S – susceptible, MR – medium resistant

Molecular analysis

The presence of *Lr37* in single F_3 plants of the cross Boka/Renan tested in the greenhouse for leaf rust reaction was also determined by a molecular marker. Table 4 shows the results of the tests with plants inoculated both with leaf and stem rust isolates. A positive reaction was obtained with DNA from all plants that were resistant or medium resistant to stem rust. The same plants were either resistant or susceptible to leaf rust. The plants resistant to stem rust and susceptible to leaf rust are supposed to be heterozygous for the translocation from *Aegilops ventricosa*. No hybridization of the marker DNA with DNA from plants susceptible both to stem and to leaf rust was observed. As shown in Figure 1, Boka gave negative, Renan and NIL *Lr37* positive reactions and so did the plants that were resistant to leaf rust in the greenhouse. Susceptible plants gave a negative response with the marker, which also suggests that these plants were homozygous susceptible.

Field experiments

Three field experiments were carried out with F_3 progenies (Table 5). In the field experiments cultivar Renan was scored as R (resistant) with disease severity up to 10%, Boka was scored S(MS) (susceptible, medium susceptible) with disease severity above 30%. F_1 generation of the cross displayed reactions similar to those of Boka with slightly lower disease severity. In F_2 generation of the cross Boka/Renan forty plants were scored in the field; F_3 progenies were developed from individual plants and scored in the field next year. Whereas the fit of F_2 plants with 1 R:3 S segregation ratio was poor (χ^2 = 2.2, P = 0.20–0.05), the classification of forty F_3 progenies derived from the same plants fitted well with the expected ratio 1 R:2 segregating:1 S progenies (χ^2 = 0.15, P = 0.95–0.80) (experiment A, Table 5). Probably four F_2 plants scored as MS were misclassified and actually belonged to the resistant group. In the other two experiments a part of F_3 progenies derived from not classified F_2 plants (76 progenies) from the cross Boka/Renan was scored in 2002, another part (67 progenies) was scored in 2003. In the field trials a fit (experiment B) or a better fit (2003 experiment) with the expected ratio for one gene was obtained when the number of susceptible and segregating progenies was pooled. Natural field infection did not enable to do safe discrimination between segregating and homozygous susceptible progenies. It was also difficult to distinguish medium susceptibility governed by *Lr13* from full susceptibility.

A decisive effect of *Lr37* on resistance in the field was also confirmed by repeated selection of the most resistant plants in field trials. The selection was carried out from resistant progenies since F_3 generation for two next generations. Finally 28 progenies most resistant in the field were tested in the greenhouse. All showed resistant reactions

Table 5. Segregation for the reaction to *Puccinia triticina* of the cross Boka/Renan – field reaction (Boka S(MS), Renan R)

Generation	Number of plants/progenies*				χ^2	P
	R	S (MS)	segregating	total	1:3 / 1:2:1*	
F_2 (2001)	6	34	–	40	2.2	0.20–0.05
F_3* (2002) experiment A	10	11	19	40	0.15	0.95–0.80
F_3* (2002) experiment B	12	31	33	76	10.82	< 0.01
S(MS)+Seg. pooled	12	64	–	76	3.44	0.2–0.05
F_3* (2003 experiment)	15	22	30	67	2.19	0.5–0.2
S(MS)+Seg. pooled	15	52	–	67	0.24	0.8–0.5

R – resistant, S – susceptible, MR – medium resistant

Experiment A conprised progenies of plants scored in F_2 generation, experiment B progenies of plants selected at random

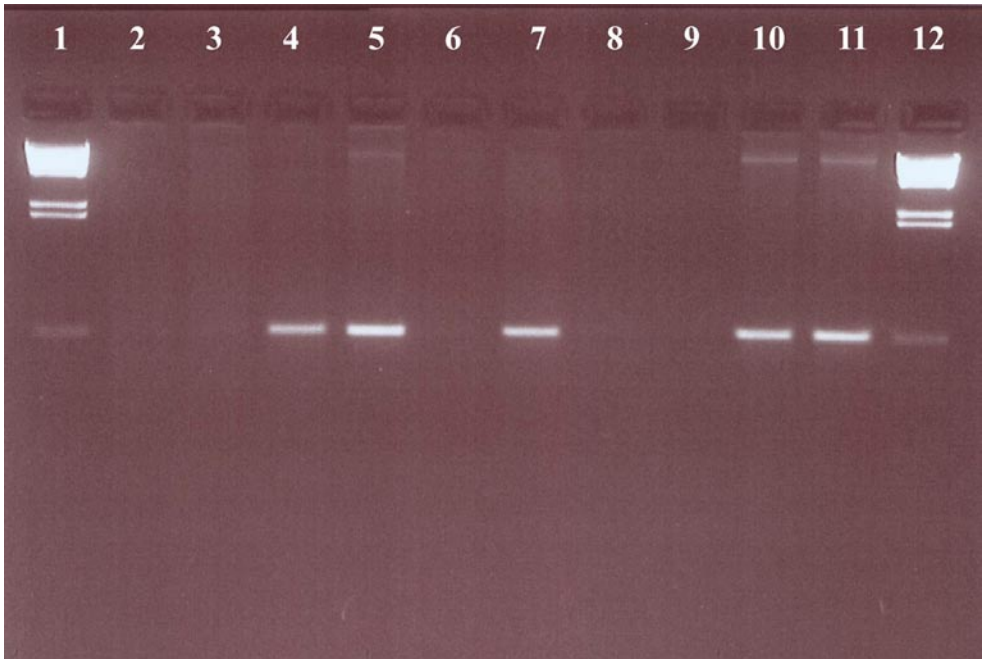

Figure 1. PCR amplification profile of homozygous F_3 plants of the cross Boka/Renan, cvs. Renan, Boka and NIL *Lr37* to the molecular marker according to Seah *et al.* (2001)

1 and 12 ladder, 2,3,8,9 susceptible plants, 4 and 5 resistant plants to leaf rust, 6 Boka, 7 Renan, 10 and 11 NIL *Lr37*

(IT ;1) to isolate 333 characterised by avirulence to *Lr37*. This test was repeated at a higher greenhouse temperature with similar results but IT was higher (IT 2–2+). The effect of temperature on the expression of *Lr37* was already described earlier by McIntosh *et al*. (1995). The same progenies were tested by a molecular marker. Positive reactions in all of them proved the presence of *Lr37*.

DISCUSSION

Leaf rust resistance gene *Lr37* is a gene for adult plant resistance though leaf rust pathotypes exist that are avirulent to *Lr37* already at the seedling stage. Genes *Yr17* and *Sr38* are effective to avirulent pathotypes both at the seedling and at the adult plant stage. Virulence to all three resistance genes has already been described. The gene *Yr17* for yellow rust resistance was overcome first in Western Europe (U.K., Denmark in 1994) where wheat cultivars with that gene were grown on a large area (Hovmøller 2001). In the years 1999–2000 virulence to *Yr17* was found also in the Czech Republic (Bartoš unpublished), however the bulk of urediospores from our yellow rust collection was avirulent on cv. Renan both at the seedling and at the adult plant stage. Virulence to *Sr38* was determined in one of the seven tested stem rust pathotypes originating from the former Czechoslovakia (Bartoš *et al*. 2004a). Data on virulence to *Lr37* in the COST 817 ring test were summarized by Mesterházy *et al*. (2001). Whereas the number of isolates virulent to *Lr37* at the seedling stage was high (over 95%) in the field tests under natural infection, disease severity did not exceed 10% except in Romania (Fundulea), where the score 50 MR–MS on NIL *Lr37* was reported. Csösz *et al.* (2000) presented data on disease severity from the period 1995–1999 at six locations in Hungary. Disease severity on NIL *Lr37* was low except Szeged, where it was scored 80 MR in 1996 and 60 VR–MR in 1999. According to Bayles (2003 personal communication) in the U.K. cultivars possessing *Lr37* still display resistance in the field. Mesterházy *et al*. (2000) reported disease severity 35MR on *Lr37* in Aberystwyth in 1999. In the Czech Republic registered cultivars possessing *Lr37* (Apache, Bill, Caphorn, Clarus, Clever, Corsaire, Rapsodia, Rheia) belong to the most resistant ones (Bartoš *et al*. 2004b). In France Robert *et al*. (2000) compared the adult plant leaf rust resistance of several cultivars that had positive

reactions in the molecular *SC-Y15* test indicating the presence of *Lr37*. Whereas disease severity on NIL *Lr37* was scored as 30%, it was scored as 0% on most other cultivars including Renan. Other leaf rust resistance genes seemed to be also involved in the expression of resistance of those cultivars. For this reason the breakdown of resistance conditioned by *Lr37* can be masked by other genes for leaf rust resistance that are still effective either alone or in combination with *Lr37*. E. g. Stepień *et al*. (2004) reported *Lr13* for cv. Apache and *Lr10* and *Lr13* for cv. Clever in addition to *Lr37*. In cv. Renan *Lr14a* was postulated by Goyeau & Park (1997) besides *Lr37*. The line VPM1, one of the parents of cv. Renan, was supposed to possess also additional gene(s) for adult plant resistance (Dyck and Lukow 1988). In addition to rust resistance cv. Renan possesses powdery mildew resistance gene *Pm4b*. It also has *Pch1* for eyespot resistance (Lind 1999). Resistance to Fusarium head blight was described by Bai and Shaner (2004) and observed also by Chrpová (personal communication). Resistance to preharvest sprouting has been recorded as well (Groos *et al.* 2002). In the tests by Nowotna *et al*. (2003) with several cultivars Renan was characterized by the highest protein and gluten contents, gluten index and amount of soluble carbohydrates as well as medium amylolytic activity and lowest crude fibre. Starch isolated from cv. Renan belonged to the best class A and was characterized by the highest molecular mass. In EVIGEZ (2007) raw protein content for cv. Renan was recorded between 12.7% and 13.8%, wet gluten between 30.1% and 35.0%. BAC library of cv. Renan was developed for genetic studies. Cv. Renan was used as one parent in the production of hybrid wheat Hyno-Kalia and Hyno-Rista. Renan was also tested in the Czech State Variety Trials in the 1990s but has not been registered. Susceptibility to septoria leaf blotch is a negative trait of cv. Renan.

Our greenhouse as well as field experiments showed the presence of one leaf rust resistance gene for high resistance in cv. Renan. That gene behaved as recessive or partially dominant and F_2 generation segregated at the ratio 1 R:3 S. Unexpected was segregation within some heterozygous F_3 progenies of the cross Boka/Renan at the ratio 1 S:3 R. However, F_3 progenies counted 15–20 plants, which may not have been enough for the safe discrimination of dominance from partial dominance. Partially dominant or recessive behaviour of the gene *LrVPM* (= *Lr37*) was also described by Dyck and Lukow (1988). Gene for stem rust resistance *Sr38*, which is also located on the translocation from *Aegilops ventricosa* and therefore closely linked with *Lr37*, was dominant. Greenhouse results validated by a molecular marker verified that the studied resistance was conditioned by the resistance gene *Lr37*. It governed seedling resistance to leaf rust isolate 333 as well as adult plant resistance to the field leaf rust population.

The presence of *Lr14a* in cv. Renan postulated by Goyeau *et al.* (1997) and recently by Pathan and Park (2006) was not confirmed. The gene *Lr14a* is located in chromosome 7B, whereas *Lr37* in chromosome 2A. If both genes were present in cv. Renan, the segregation of two independent genes would be expected in F_2 generation of the cross Boka/Renan, i.e. 13 R:3 S for one dominant and one recessive gene or 7 R:9 S for two recessive genes. In the F_3 generation ratio 7 homozygous resistant progenies, 8 segregating and 1 homozygous susceptible progeny should be found. That was not the case in our experiments. Reactions of two leaf rust isolates that were avirulent to *Lr14a* but virulent to *Lr37* and Renan do not support the postulation of *Lr14a* in Renan either. Epistasis of *Lr14a* over *Lr37* can be expected and hence the reaction of Renan should be similar to that of *Lr14a*. However, the seed of cv. Renan used in our trials was not identical with the seed used by the authors mentioned above. Environmental variability of *Lr14a* is high and the host genetic background also affects the expression of *Lr14* (McIntosh *et al.* 1995). This may have caused different results.

Our results do not exclude the presence of other genes for leaf rust resistance in cv. Renan. The results published by Robert *et al.* (2000), who recorded higher resistance in cv. Renan than in NIL *Lr37*, also suggest a more complex genetic background of the leaf rust resistance of cv. Renan. In our field nursery cv. Renan usually showed slightly higher resistance to the leaf rust than NIL *Lr37*. Minor genes modifiers are supposed to be responsible for the enhanced resistance of Renan. However, leaf rust resistance gene *Lr37* seems to play the decisive role in the field resistance of cv. Renan.

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