Theor Appl Genet (2000) 100:419–431

M.M. Messmer · R. Seyfarth · M. Keller
G. Schachermayr · M. Winzeler · S. Zanetti
C. Feuillet · B. Keller

Genetic analysis of durable leaf rust resistance in winter wheat

Received: 1 July 1999 / Accepted: 30 July 1999

Abstract Quantitative resistance that delays the epidemic development of leaf rust in wheat is an important source for durable resistance breeding. The Swiss winter wheat variety 'Forno' shows a high level of quantitative resistance against leaf rust. This resistance has been effective for more than 10 years and can therefore be considered to be durable. In order to map quantitative trait loci (QTL) for durable leaf rust resistance we analysed 204 F_5 recombinant inbred lines (RILs) of the cross between the winter wheat 'Forno' and the winter spelt 'Oberkulmer' for their level of leaf rust resistance (LR) and leaf tip necrosis (LTN) in four different environments. Both traits showed a continuous distribution and were significantly correlated (r=–0.5). Across environments we detected 8 QTL for leaf rust resistance (6 inherited from 'Forno') and 10 QTL for the quantitative expression of LTN (6 inherited from 'Forno'). Of the 6 QTL responsible for the durable leaf rust resistance of 'Forno', 1 major QTL coincided with a thaumatin locus on 7BL explaining 35% of the phenotypic variance. Four QTL for LR coincided with QTL for LTN. At these loci the alleles of 'Forno' increased the level of resistance as well as the extent of LTN, indicating pleiotropy.

Communicated by G. Wenzel

M.M. Messmer · M. Keller · G. Schachermayr · M. Winzeler
S. Zanetti
Swiss Federal Research Station for Agroecology
and Agriculture (FAL) Zürich-Reckenholz, Reckenholzstr. 191,
CH-8046 Zürich, Switzerland

R. Seyfarth · C. Feuillet · B. Keller (✉)
Institute of Plant Biology, University of Zürich, Zollikerstr. 107,
CH-8008 Zürich, Switzerland
e-mail: bkeller@botinst.unizh.ch
Fax: +41-1-634 82 04

M. Keller
Institute of Plant Sciences,
Swiss Federal Institute of Technology (ETH), Universitätsstr. 2,
CH-8092 Zürich, Switzerland

Present address: M.M. Messmer
Pharmaceutical Institute, University of Basel, Benkenstr. 254,
CH-4108 Witterswil, Switzerland

Key words *Triticum aestivum* · QTL · Leaf rust · Durable resistance · Leaf-tip necrosis

Introduction

Leaf rust caused by the pathogen *Puccinia recondita* Roberge ex Desmaz. f. sp. *tritici* (Eriks. & E.Henn) is world wide a major disease of wheat (*Triticum aestivum* L.). Susceptible wheat varieties suffer regularly yield reductions of 5–15% or greater depending on the stage of crop development when the initial rust infection occurs (Kolmer 1996). There are two main breeding strategies to improve leaf rust resistance: pyramidization of the major resistance genes (*Lr* genes) conferring complete resistance and/or the accumulation of minor resistance genes conferring quantitative resistance. Many efforts have been undertaken to introgress *Lr* genes into wheat breeding material (for review see McIntosh et al. 1995). However, the resistance conferred by a single gene is frequently overcome by the appearance of virulent races in the pathogen population within a short period of time. To obtain a more durable resistance, quantitative resistance, so-called partial or slow rusting resistance, is preferred, in which the infection is not completely stopped but the spread of the disease is delayed. In general, slow rusting wheat has longer latent periods, fewer uredinia, and smaller uredinia size at 10–14 days after inoculation with leaf rust than susceptible wheat lines (Kolmer 1996).

Several genetic studies have been performed in order to determine the inheritance of slow rusting in wheat (reviewed by Geiger and Heun 1989). In most of these studies transgressive segregation for leaf rust resistance was found as well as partial dominance for susceptibility. The inheritance of resistance was attributed to only one to three genes with predominantly additive gene action and, in some crosses, also with significant epistatic effects (Geiger and Heun 1989). A genetic analysis for latent period of *Puccinia recondita* in wheat performed by Shaner et al. (1997) provided evidence that four genes

with unequal and epistatic effects controlled the latent period, whereas VanderGaag and Jacobs (1997) found at least five genes to be involved in the prolongation of the latent period. The leaf rust resistance conferred by the adult-plant resistance gene *Lr34*, i.e., longer latent period, fewer uredinia and smaller uredinia size, matches the description of slow rusting (Kolmer 1996). Since many wheat lines characterized for slow rusting were derived from sources of *Lr34* containing lines it is very likely that *Lr34* was segregating together with other genes in these studies (Kolmer 1996). The *Lr34* gene is pleiotropic or closely linked with leaf tip necrosis caused by the major gene *Ltn* located on chromosome 7DS (Singh 1992) that was used for indirect selection of leaf rust resistance. Singh et al. (1998) studied the inheritance of adult plant resistance of the spring wheat variety 'Pavon 76' and found another gene involved in slow rusting resistance, designated as *Lr46*, which was located on chromosome 1B.

The dissection of quantitative traits into Mendelian factors of inheritance, so-called quantitative trait loci (QTL), provides a powerful tool for identifying genes with minor effects and enables the identification of the whole set of genes important for the resistance reaction in a specific cross. William et al. (1997) developed markers associated with QTL conferring durable leaf rust resistance to the variety 'Parula', which contains the *Lr34* gene and at least two additional genes for slow rusting (Singh and Rajaram 1992), and found three randomly amplified polymorphic DNA (RAPD) markers associated with leaf rust resistance in the field: two were located on chromosome 7BL and one hybridized to chromosomes 1BS and 1DS. In addition, leaf tip necrosis assessed as a monogenic trait could explain 20–25% of the phenotypic variation for leaf rust resistance in this cross over 2 years. Nelson et al. (1995b) analysed recombinant inbred lines of a cross between synthetic wheat and 'Opata' for leaf rust resistance in the field. They found two restriction fragment length polymorphic (RFLP) marker loci that were associated with leaf rust resistance in the field: *Xwg834* (positive allele from 'Opata', which contains the *Lr34* gene) located on chromosome 7DS and *Xbcd152* (positive allele from the synthetic wheat) located on chromosome 2BS (Nelson et al. 1995a). Both loci explained together 45% of the phenotypic variance (Nelson et al. 1995b). This population was further analysed by Nelson et al. (1997) in order to identify chromosomal regions conferring leaf rust resistance in seedling and adult plant stages and to examine their interaction. Several chromosomal regions were found to influence adult plant resistance in four field experiments using different pathotypes, but the only consistent region was on 7DS carrying the *Lr34* adult-plant resistance gene. Under conditions of natural infection, Faris et al. (1999) reported a strong association between leaf rust resistance and a cluster of defense response genes located on 7BL.

Little is known about the genetic basis of quantitative leaf rust resistance in the European winter wheat breeding material. Winzeler et al. (1995) tested European wheat lines for their adult plant leaf rust resistance in the field and their seedling resistance in the growth chamber. While 50% of the spring wheat lines with a high level of field resistance also showed seedling resistance (indicating the presence of major genes), less than 20% of the winter wheat lines with sufficient adult plant resistance showed seedling resistance. The Swiss variety 'Forno' (pedigree: 'NR72837×Kormoran') has been grown in Switzerland for more than 10 years (since 1986), and for 5 of these years on more than 5% of the wheat acreage, without losing its high level of resistance against leaf rust. Therefore, 'Forno' can be considered as being durable resistant according to the definition of Johnson (1983). Moreover, the high level of adult plant resistance shown by 'Forno' is effective across Europe: in the European winter wheat nursery of COST 817 'Forno' was tested for more than 3 years in up to nine different European countries and only once did it show moderate susceptibility (Winzeler, unpublished data). Recently, a genetic map containing 230 marker loci was constructed for F_5 recombinant inbred lines (RILs) of a cross between the Swiss winter wheat 'Forno' and the Swiss winter spelt (*Triticum spelta* L.) 'Oberkulmer' (Messmer et al. 1999). We have used this population to study the genetic basis of leaf rust resistance in 'Forno' The objectives of the study reported here were to identify and localize individual genes (QTL) responsible for the expression of resistance against leaf rust observed under field conditions and to elucidate the genetic basis of leaf tip necrosis in European winter wheat material and its phenotypic correlation to leaf rust resistance. The ultimate goal of the QTL analysis is to develop tools that are useful for marker-assisted selection (MAS) in practical breeding programs towards more durable resistance.

Materials and methods

Plant materials

For the genetic analysis of leaf rust resistance we used a cross between the Swiss winter wheat (*Triticum aestivum* L.) var 'Forno' and the Swiss winter spelt (*Triticum spelta* L.) var 'Oberkulmer' (Messmer et al. 1999; Keller et al. 1999a,b). 'Forno' has a high level of resistance against leaf rust and shows leaf tip necrosis, whereas 'Oberkulmer' is medium susceptible to leaf rust and has no leaf tip necrosis. Both parents are not known to carry any *Lr* genes according to pedigree data.

Field trials

The 226 RILs of 'Forno×Oberkulmer' were cultivated in four different environments: in 1995 at Ruemlang (Ru95) and Reckenholz (Sn95) and in 1996 at two locations in Reckenholz (Sn96 and Re96). The 226 RILs were grown together with three replicated entries of the parental lines 'Forno' and 'Oberkulmer', the F_1 'Forno×Oberkulmer' (not included in Re96), and 17 standard varieties in a rectangular lattice design with two replications and 10 genotypes per incomplete block. The material was sown as naked kernels in 5-row plots (200 kernels per 2.5 m^2) except for the trial Re96, where the material was sown as a 6-row drill plot (6 m^2) with 350 naked seeds/m^2. Lodging was prevented by mounting a plastic net over the plots below the flag leaves in

Ru95, Sn95, and Sn96. In Re96 we planted an isolation track of a mixture of the Swiss winter wheat varieties 'Arina' and 'Bernina' between the experimental plots in order to prevent severe lodging. These isolation tracks, but not the 250 genotypes, were treated with 0.5 l/ha of the growth regulator Moddus (Novartis, Switzerland) at growth stage DC 33 (Zadoks et al. 1974). In all trials oat slugworms were controlled by spraying 1.5 l/ha Zolone (Maag, Switzerland) at growth stages 50–55. Foot rot diseases were prevented by applying 1 l/ha of Tiptor (Maag, Switzerland) at DC 25 (6 weeks before booting) in Sn95 and Sn96 and 1 l/ha of Sportak (Bayer, Germany) at DC 31 in Re96.

The field trial in Re96 was artificially inoculated with leaf rust, whereas natural infection occurred at the other three locations. For the artificial infection, seedlings of the highly leaf rust-susceptible varieties 'Arina' and 'Bernina' were grown in Jiffy pots in the green-house. When the first leaf was fully emerged and the second leaf had appeared seedlings were sprayed with a mixture of talcum and leaf rust urediospores from 16 selected isolates collected in Switzerland. The infected seedlings were kept at high humidity (mist) in the greenhouse. The isolates were selected for virulence against known monogenic seedling resistance genes. The mixture of isolates was virulent on *Lr2a*, *Lr2b*, *Lr2c*, *Lr3a*, *Lr3b*, *Lr10*, *Lr11*, *Lr14a*, *Lr14b*, *Lr15*, *Lr16*, *Lr17*, *Lr18*, *Lr20*, *Lr21 Lr23*, *Lr26*, *Lr27+Lr31*, *Lr29*, *Lr30*, *Lr32*, *Lr33*, *Lr44*, but avirulent on *Lr1*, *Lr9*, *Lr19*, *Lr24*, *Lr25*, *Lr28*, and *Lr38*, because such isolates occur only at low frequencies in Switzerland (Winzeler et al. 1995). After successful inoculation the seedlings were cultivated outside the greenhouse and 3 weeks later planted in the field to start leaf rust infection. To guarantee a regular infection with leaf rust in the whole field, we planted the leaf rust-infected seedlings in the isolation tracks of the 'Arina'/'Bernina' mixture every 5 m on both sides of the experimental plots in early May at growth stage DC 32 (Zadoks et al. 1974).

Phenotypic assessment of leaf rust and leaf-tip necrosis

Leaf rust resistance (LR) was recorded for the 226 RILs of 'Forno×Oberkulmer' and the parental lines on a scale from 1 (no pustules=resistant) to 9 (leaf area totally covered with pustules=highly susceptible) on a field plot basis. In the field trials Ru95, Sn95, and Sn96 there was a medium to low pressure of leaf rust due to natural infection. Therefore, the rating could only be performed once during the grain filling period (9 July, 1 July, 6 July, respectively), with the occurrence of pustules on the flag leaf being the primary factor considered. Due to the artificial inoculation in the trial Re96 the infection pressure was much higher, and leaf rust resistance was recorded twice (27 June and 5 July), considering the total leaf area. The mean of the two assessments was taken for the leaf rust score of Re96. In all trials we assessed days until ear emergence and flowering, culm length, and the occurrence of leaf tip necrosis. Presence or absence of leaf tip necrosis was recorded during anthesis in the trial Sn95 (28 May and 19 June), Sn96 (6 June), and Re96 (5 June). In addition, the extent of leaf tip necrosis (LTN), measured in centimeters from the tip of the leaf, was recorded in the trials Ru95 (2 July), Sn96 (26 June), and Re96 (26 June).

Statistical analysis

Lattice analysis of single environments and analysis of variance (ANOVA) across environments were performed with the computer program PLABSTAT (Utz 1995). The adjusted entry means obtained from the lattice analysis were used for a combined ANOVA over environments in order to estimate the genotypic (σ^2_G) and the genotype×environment interaction ($\sigma^2_{G\times E}$) variance components. Heritability values (h^2) were based on the variance components of the ANOVA and calculated according to Hallauer and Mirando Fo (1981). To determine the genetic correlation between LR and LTN we performed a covariance analysis over three environments (Ru95, Sn96, Re96) with the original data assuming a complete block design. For the calculation of the F_5 population mean and the correlation between different traits and different environments, the parental lines and standard lines were excluded, as well as 22 F_5 RILs that had an increased level of heterogeneity/heterozygosity (>10%) or an indication of outcrossing based on the molecular data (Messmer et al. 1999). Adjusted mean values of the remaining 204 RILs of 'Forno×Oberkulmer' from single environments as well as the overall mean were used for QTL analysis.

QTL analysis was based on the genetic map constructed by Messmer et al. (1999) spanning 2469 cM. The average marker density for the QTL analysis was 13.6 cM with at least 2 marker loci per chromosome. QTL analysis was performed with the computer package PLABQTL (Utz and Melchinger 1996) based on composite interval mapping (CIM) applying the additive model. Additive effects were negative if the allele of 'Forno' increased LR score or LTN measurement and positive if the 'Forno' allele decreases the LR or LTN. For QTL detection, a LOD threshold of 3.0 was applied corresponding to an alpha level of 0.001 on a single locus basis and an overall alpha of 0.159 for the 159 marker intervals. The QTL position, given as centiMorgans from the top of the chromosome, was determined when the LOD score reached its maximum. A support interval with a LOD fall-off of 1.0 was given for each QTL. QTL with a non-overlapping support interval are assumed to be different. The percentage of phenotypic variance explained by a single QTL (R^2) is based on the partial correlation of a putative QTL with the observed data adjusted for the selected cofactors. In the simultaneous fit, the cofactors are ignored, and only the detected QTL and their estimated positions were used for multiple regression to obtain the final estimate of the additive effects (a) and total percentage of phenotypic variation that can be explained by the QTL. In the simultaneous fit we also estimated the squared partial correlation coefficient (part. R^2) of individual QTL, which is obtained by keeping all other detected QTL as fixed effects. The occurrence of QTL×QTL interactions was tested for significance by adding digenic epistatic effects to the additive effects in the model. The QTL×environment interaction for leaf rust resistance was estimated by fitting a model to the adjusted entry means of each environment which included all QTL detected in the analysis across environments, as described by Bohn et al. (1996).

Results

Phenotypic segregation

The natural infection of leaf rust started 3 weeks after anthesis and resulted in medium infection pressure in Ru95 (average LR score=3.0), Sn95 (average LR score=2.6), and Sn96 (average LR score=2.8). With the artificial inoculation, leaf rust infection started 1 week after anthesis and caused higher levels of disease scores (average LR score=4.5). In each environment the parental lines showed significant (P<0.05) differences in leaf rust resistance. Leaf rust scores of replicated entries of 'Forno' varied from 1 to 2, i.e. 'Forno' showed in all four experiments no or only few pustules with uredinia spores. On the other hand, replicated entries of 'Oberkulmer' varied from 3 to 4 for leaf rust score in Ru95, and from 5 to 8 in Sn96, but were consistently 4 and 5.5 in Sn95 and Re96, respectively.

The qualitative assessment of leaf tip necrosis (assuming a monogenic trait) was difficult and very much dependent on the time of assessment. Only 63% of the genotypes were classified identical for the presence or absence of leaf tip necrosis for the first and second rating

Table 1 Leaf rust resistance score (LR 1–9) and leaf-tip necrosis (LTN, cm) of the parental wheat line 'Forno', the spelt line 'Oberkulmer', the F_1, and the 204 F_5-RILs of 'Forno×Oberkulmer' for the four environments (Ru95, Sn95, Sn96, Re96) based on adjusted entry means (*SD* standard deviation)

Environments	Traits	Forno	Oberkulmer	Parental mean	F_1	F_5-RILs mean	F_5-RILs SD	F_5-RILs range
Ru95	LR:	1.46	3.78	2.62	1.98	3.03	0.91	0.9–6.0
	LTN:	3.01	0.16	1.59	2.90	1.81	1.62	–0.5–8.2
Sn95	LR:	1.06	4.12	2.59	2.01	2.61	1.27	0.8–7.2
	LTN:	–	–	–	–	–	–	–
Sn96	LR:	1.36	5.43	3.40	0.98	2.79	1.28	0.4–6.3
	LTN:	6.63	0.00	3.32	4.08	2.68	2.66	–0.3–8.1
Re96	LR:	1.51	5.46	3.49	–	4.36	1.54	1.4–8.1
	LTN:	6.84	0.17	3.51	–	2.14	1.08	–0.2–6.5
Average	LR:	1.30	4.70	3.00	2.05	3.19	1.14	1.1–6.1
	LTN:	5.49	0.10	2.80	3.47	2.21	1.92	0.0–7.1

in Sn95 and only 72% were classified identical when the two replications were compared (r=0.54). Therefore, leaf tip necrosis was assessed as a quantitative trait by measuring the extent of leaf tip necrosis in centimeters. Both the qualitative and the quantitative assessment of leaf tip necrosis were significantly (P<0.01) correlated with r=0.8 in the experiments Sn96 and Re96, but the quantitative assessments resulted in a better correlation between replications (0.80<r>0.86). Because of the better reproducibility all data presented here refer to leaf tip necrosis measured in centimeters (LTN). The extent of LTN differed strongly in different years. Replicated entries of 'Forno' varied from 2 to 4 cm in 1995 (Ru95) and from 5 to 8 cm in 1996 (Sn96, Re96), while 'Oberkulmer' showed a maximum of 1 cm of leaf tip necrosis in the three experiments.

Across all experiments the parental lines 'Forno' and 'Oberkulmer' differed significantly (P<0.05) for LR (1.3 vs. 4.7) and LTN (5.5 vs. 0.1 cm). The F_1 hybrid of 'Forno×Oberkulmer' had a significantly (P<0.05) lower LR score (i.e., more resistant) than the mean of the parental lines, indicating the presence of dominance effects for leaf rust resistance, whereas the amount of LTN did not deviate significantly from the parental mean (Table 1).

Each field trial was analysed as a lattice design, resulting in an improved efficiency of 103% (Ru95) to 124% (SN96) for LR and of 100% (Re96) to 123% (Ru95) for LTN compared to analysis as a complete block design (100%). Due to block effects the adjusted entry mean of some genotypes became smaller than the original scale for LR and LTN (Table 1). In each trial the 204 F_5-RILs of 'Forno×Oberkulmer' showed transgressive segregation for both traits (Table 1). Across all experiments these ranged from a score of 1.1 to 6.1 for LR (Fig. 1A) and from 0.0 to 7.1 cm for LTN (Fig. 1B). No consistent deviation was found between the mean of the 204 RILs and the parental mean over all environments for LR and LTN (Table 1). The adjusted entry means of the 204 RILs were significantly (P<0.01) correlated between the different experiments for LR (0.72<r<0.80)

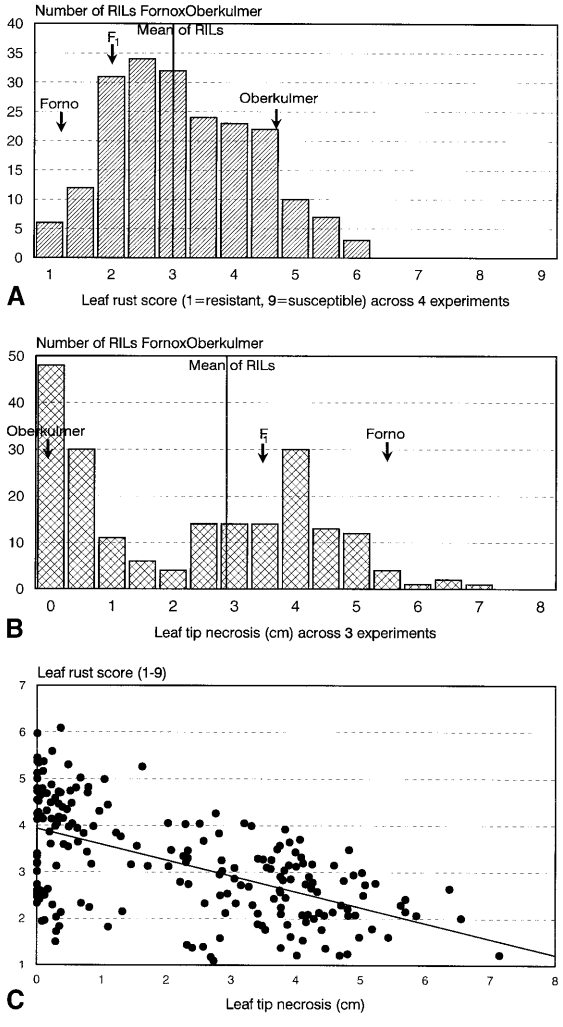


Fig. 1A–C Phenotypic distribution of the 204 RILs from the cross 'Forno×Oberkulmer', their parental lines 'Forno' and 'Oberkulmer', and their F_1 hybrid for leaf rust resistance (**A**) and leaf-tip necrosis (**B**) across the different environments and their phenotypic correlation (**C**)

Table 2 Identification of significant (LOD>3.0) QTL for leaf rust resistance (LR), their chromosomal location, support interval, flanking markers, and explained phenotypic variance (R^2) based on composite interval mapping of the adjusted means of 204 RILs of 'Forno×Oberkulmer' for the four different environments. Partial regression coefficients (part. R^2) and additive effects (LR score) are obtained from the simultaneous fit of all putative QTL by multiple regression

QTL position for leaf rust resistance			Ru95			Sn95			Sn96			Re96		
Chromosome	Centi-Morgans [support interval]	Flanking markers	R^2 (%)	Part. R^2 (%)	Additive effect (score)	R^2 (%)	Part. R^2 (%)	Additive effect (score)	R^2 (%)	Part. R^2 (%)	Additive effect (score)	R^2 (%)	Part. R^2 (%)	Additive effect (score)
1BS	0 [0–6]	*CD9b* – *Xpsr593a*							11.5	1.4	+0.13			
1BS	32 [30–34]	*Xpsr949* – *Xgwm18*	15.0	9.8	+0.23							9.2	4.9	+0.30
2B	110 [98–118]	*Xglk400* – *Xpsr924*	8.2	6.4	–0.19									
3A	54	*Xpsr598* – *Xpsr570*	20.3	12.9	+0.30									
	64 [48–68]	*Xpsr570* – *Xpsr543*							8.1	5.3	+0.27			
3B	36 [34–40]	*Xpsr919a* – *Xpsr1101b*				9.3	3.9	–0.24						
4B	34 [20–52]	*Xpsr921* – *Xpsr953b*				12.6	4.8	+0.28	9.6	4.9	+0.27	6.9	0.9	+0.13
4DL	106;112 [92–114]	*Xglk302b* – *Xpsr1101a*				11.6	4.3	+0.24	8.7	2.9	+0.22	19.8	12.1	+0.46
5A	40 [30–50]	*Xpsr945a* – *Xglk424*										7.7	13.3	+0.51
5B	134 [128–142]	*Xpsr580b* – *Xpsr143*	14.6	6.4	+0.20									
5DL	2 [0–32]	*Xpsr906a* – *Xpsr580a*							9.1	11.2	–0.42			
7B	28 [14–44]	*Xpsr952* – *Xgwm46*				7.6	3.5	–0.30						
7B	88	*Xpsr350* – *Pwir232b*							22.7	19.9	+0.55			
	96;90;92 [84–104]	*Xpsr593c* – *Xpsr129c*	33.6	22.5	+0.48	19.3	21.8	+0.62				31.1	21.7	+0.72
7B	142 [140–150]	*Xglk750* – *Xmwg710a*	9.3	4.8	+0.16									
	Phenotypic variance explained simultanously (%)		51.7			27.6			36.1			38.3		

Table 3 Identification of significant (LOD>3.0) QTL for leaf tip necrosis (LTN), their chromosomal location, support interval, flanking markers, and explained phenotypic variance (R^2) based on composite interval mapping of the adjusted means of 204 RILs of 'Forno×Oberkulmer' for the three different environments. Partial regression coefficients (part. R^2) and additive effects (LTN in cm) are obtained from the simultaneous fit of all putative QTL by multiple regression

QTL position for leaf tip necrosis				Ru95			Sn96			Re96		
Chr.	Centi-Morgans	Support interval	Flanking markers	R^2 (%)	Part. R^2 (%)	Additive effect (cm)	R^2 (%)	Part. R^2 (%)	Additive effect (cm)	R^2 (%)	Part. R^2 (%)	Additive effect (cm)
1A	94	88–94	*Xpsr1201b* – *Xpsr941*	7.2	2.7	+0.22						
1BS	36	32–36	*Xgwm18* – *Xglk483*							7.5	7.2	+0.45
2A	0	0–6	*Xpsr958* – *Xpsr566c*							6.8	4.0	+0.32
2B	184	176–184	*Xpsr956a* – *Xglk610a*	7.1	4.6	+0.28						
3A	10	0–24	*Xpsr304* – *Xpsr598*	13.6	8.0	+0.47						
3A	146;150	138–160	*Xpsr936* – *Xpsr1203a*	9.4	4.3	–0.30				8.5	6.1	–0.41
3B	2	0–8	*Lrk10b* – *Xpsr1196b*	13.3	0.3	–0.08						
3B	24	12–32	*Xpsr907* – *Xglk538*							7.2	6.4	+0.47
4B	34	32–42	*Xpsr921* – *Xpsr953b*	6.6	4.1	–0.28						
4B	80	74–86	*Xpsr593b* – *Xpsr1112*	9.8	5.0	+0.31						
4DL	106;112	98–114	*Xglk302b* – *Xpsr1101a*	27.2	26.3	–0.91	20.9	6.8	–0.86	13.1	13.5	–0.61
5A	0	0–4	*Xpsr549* – *Xglk163a*	14.2	11.5	–0.43						
5A	76	64–86	*Xglk424* – *Xpsr912*				7.8	0.5	+0.22			
5B	70	68–76	*Xglk163b* – *Xpsr426*	9.4	3.5	+0.25						
5DL	14	0–36	*Xpsr906a* – *Xpsr580a*				7.7	5.6	+1.01			
7B	142;146	138–164	*Xglk750* – *Xmwg710a*	7.7	6.9	–0.36				6.8	5.3	–0.44
7S	74	70–76	*Xglk658* – *Xpsr938*	7.6	7.3	+0.38						
	Phenotypic variance explained simultanously (%)			48.4			10.3			33.3		

and for LTN ($0.79<r<0.90$). For both traits we found a highly significant ($P<0.01$) genotype×environment interaction, but the magnitude of the genotype×environment variance components was small ($\sigma^2_{G\times E}=0.18$ for LR and $\sigma^2_{G\times E}=0.65$ for LTN) compared to the highly significant ($P<0.01$) genotypic variance components for LR ($\sigma^2_G=1.32$) and LTN ($\sigma^2_G=3.28$). This resulted in high heritability values of 0.94 for LR and 0.92 for LTN. Averaged over all trials, the 204 RILs showed almost normal distribution for LR (right skewed and platykurtic with $P<0.10$), while LTN showed a bimodal distribution (Fig. 1B). Figure 1C illustrates the highly significant ($P<0.01$) rank correlation of $r=-0.53$ between LR and LTN. Genotypes with strong leaf tip necrosis (LTN >5 cm) were highly resistant (LR <3), whereas genotypes with little expression of leaf tip necrosis showed a large range of LR scores. The genotypic correlation based on covariance analysis between LR and LTN was –0.49. Although there was a large variation for culm length and earliness (days until ear emergence and days till flowering), no significant correlation was found between these traits and LR or LTN.

QTL analysis for leaf rust resistance in single environments

In each experiment the cofactors were selected independently and varied from 14 to 17 selected markers for LR. With composite interval mapping we revealed 5–6 significant QTL for LR in the single experiments (Table 2) and altogether 13 different genetic regions contributing to leaf rust resistance. Positive additive effects indicate that the LR score was higher for the parental allele of 'Oberkulmer', i.e., susceptibility is inherited by the 'Oberkulmer' allele and resistance (smaller LR score) by the 'Forno' allele. In the experiment Re96 all positive alleles for leaf rust resistance were contributed by the more resistant wheat parent 'Forno', whereas in the other three experiments the more susceptible spelt parent 'Oberkulmer' also contributed one or two alleles for improved resistance. The QTL on chromosome 1BS (32 cM) and 3A were consistent across two environments, the QTL on chromosomes 4B and 4DL across three, and the QTL on 7BL (88–96 cM) was consistent across all four environments. The percentage of phenotypic variance (R^2) explained by a single QTL ranged from 6.9% to 33.6% in the covariance analysis with cofactors. The total amount of phenotypic variation for LR explained by all significant QTL in the simultaneous fit varied between 27.6% in Sn95 and 51.7% in Ru95. To illustrate the importance of each QTL in the presence of the other significant QTL (obtained by multiple regression without cofactors) we provide the square of partial regression coefficient (part. R^2) of each QTL and its additive effect in Table 2. The most important QTL in each experiment was found on 7B between 84 and 104 cM, with an partial R^2 of about 20% in each experiment. The additive effect for leaf rust score of this QTL was smallest in Ru95 (+0.48) and highest in Re96 (+0.72), where we observed the highest disease pressure.

Table 4 Identification of significant (LOD>3.0) QTL for leaf rust resistance (LR) and leaf-tip necrosis (LTN), their chromosomal location, support interval [], flanking markers, and explained phenotypic variance (R^2) based on composite interval mapping of the adjusted means of 204 RILs of 'Forno×Oberkulmer' across the different experiments. Partial regression coefficients (part. R^2) and additive effects for LR score and LTN, respectively, are obtained from the simultaneous fit of all putative QTL by multiple regression

QTL position			LR across four environments			LTN across three environments		
Chromosome	CentiMorgans [support interval]	Flanking markers	R^2 (%)	Part. R^2 (%)	Additive effect (score)	R^2 (%)	Part. R^2 (%)	Additive effect (cm)
1BS	32 [30–34]	*Xpsr949* – *Xgwm18*	10.6	3.3	+0.16			
	36 [32–36]	*Xgwm18* – *Xglk483*				8.4	7.3	+0.46
2B	120 [112–126]	*Xprs924* – *Xglk699a*	7.2	3.6	–0.17			
3A	64 [58–66]	*Xpsr570* – *Xpsr543*	13.5	7.0	+0.24	6.9	1.7	–0.22
4B	34 [22–50]	*Xpsr921* – *Xpsr953b*				6.8	3.1	–0.33
	38 [32–54]		10.0	4.1	+0.22			
4B	86 [78–88]	*Xpsr593b* – *Xpsr1112*				6.8	3.2	+0.35
4DL	110;112; [100–114]	*Xglk302b* – *Xpsr1101a*				18.0	10.5	–0.58
			9.0	8.9	+0.27			
5A	0 [0–4]	*Xpsr549* – *Xglk163a*				14.4	8.5	–0.48
5DL	16 [0–38]	*Xpsr906a* – *Xpsr580a*	8.9	6.4	–0.38			
6A	78 [62–80]	*Xpsr563a* – *Xpsr966*				7.6	2.2	+0.27
7B	74 [66–82]	*Xpsr350* – *pwir232b*				7.6	5.6	+0.54
7B	92 [88–102]	*Xpsr593c* – *Xpsr129c*	35.8	19.4	+0.47			
7B	150 [140–162]	*Xglk750* – *Xmwg710a*	12.8	7.8	+0.34	15.2	13.0	–0.99
7DS	48 [36–64]	*Xpsr160a* – *Xgwm44*				9.0	5.3	–0.58
	Phenotypic variance explained simultanously (%)		50.9			40.8		

QTL analysis for leaf tip necrosis in single environments

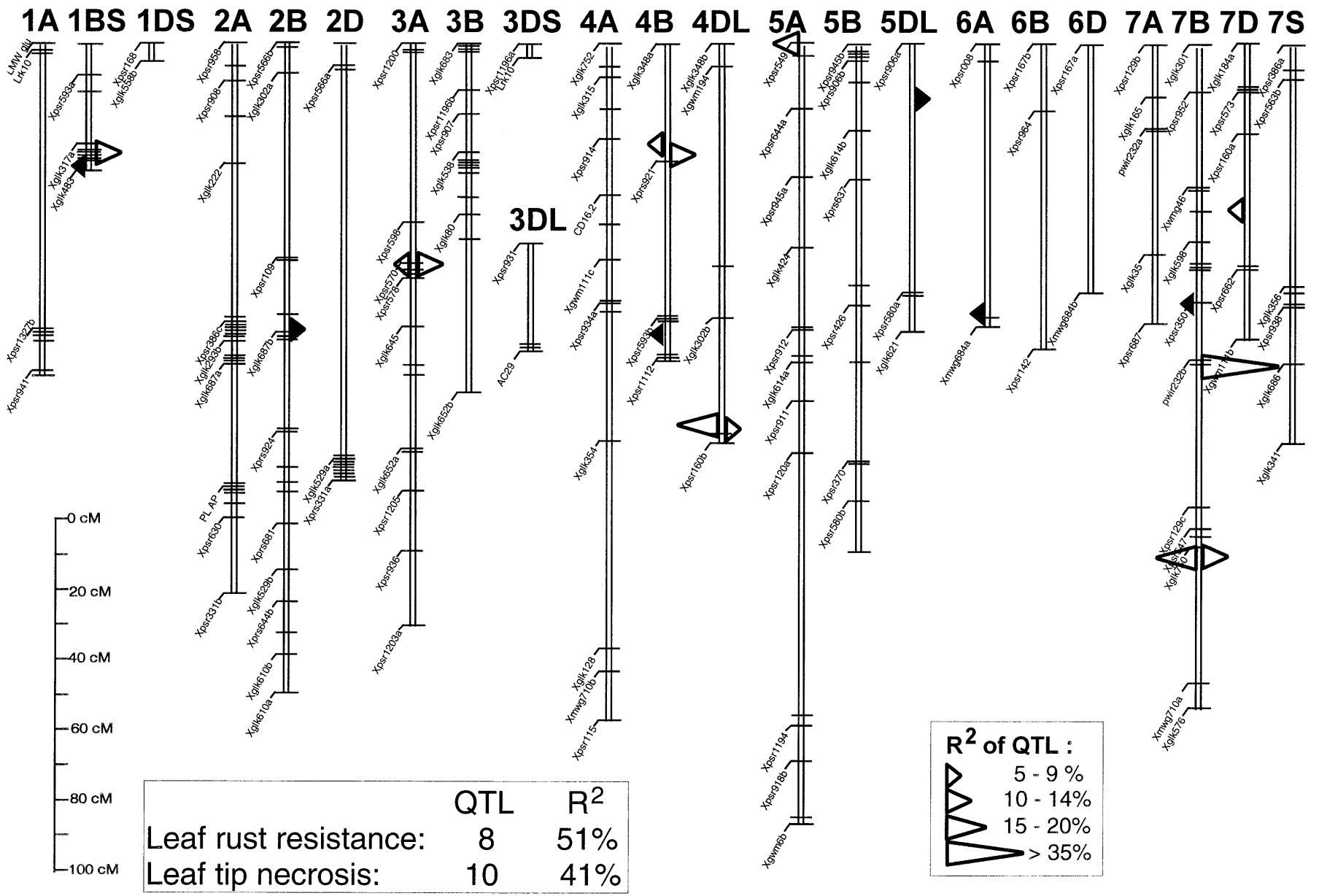
The number and location of significant QTL detected for LTN varied strongly between the three experiments (Table 3). We found 12 different QTL in Ru95, 3 QTL in Sn96, and 6 QTL in Re96 explaining in the simultaneous fit 48.4%, 10.3%, and 33.3% of the phenotypic variance, respectively. Although the spelt parent 'Oberkulmer' showed almost no leaf tip necrosis, half of the QTL detected in each experiment had a positive additive effect; i.e., the allele causing increased LTN was inherited from 'Oberkulmer'. Summarizing the data from Table 3, we detected 1 QTL on chromosome 4DL in all three environments, 2 QTL on 3A (146–150 cM) and 7B (142–146 cM) in Ru95 and Re96, and 14 genomic regions that influenced LTN in just one environment. The QTL found on 4DL (106–112 cM) explained most of the phenotypic variance for LTN in each environment (13.1% $<R^2>$27.2%).

The support interval of this QTL overlapped with the QTL found for LR with a positive additive effect in Sn96 and Re96 (Table 2). In this chromosomal region the alleles of 'Forno' increased both leaf tip necrosis and leaf rust resistance. In addition, we discovered coincidences of QTL for the two traits on chromosomes 7B (142 cM) in Ru95, 5DL (2–14 cM) in Sn96, and 1BS (32–36 cM) in Re97. While at the location on 7B the 'Forno' alleles contributed to increased LTN and LR, the 'Oberkulmer' alleles on 5D enhanced LTN and resistance. In contrast, we found that at the QTL on 1BS in Re96 the alleles of 'Oberkulmer' increased leaf tip necrosis but decreased leaf rust resistance.

QTL analysis across environments

In order to determine QTL that are important for the expression of the trait under different environmental conditions, we performed QTL analysis on the basis of the phenotypic values averaged over all environments. We detected 8 putative QTL for LR and 10 QTL for LTN across experiments explaining 51% and 41% of the phenotypic variance, respectively (Table 4). Five of the QTL for LR had overlapping support intervals with QTL for LTN (Fig. 2). At 4 genomic regions 'Forno' contributed towards improved resistance associated with increased leaf tip necrosis, while on 1BS (32–36 cM) the 'Forno' allele improved resistance but reduced the amount of leaf tip necrosis. Although 'Oberkulmer' was more susceptible to leaf rust (LR=4.7) and had only a small amount of leaf tip necrosis (LTN=0.1 cm), 'Oberkulmer' alleles contributed towards increased resistance at 2 putative QTL and towards an increased level of leaf tip necrosis at 4 putative QTL. A summing up the additive effects of the 'Forno' alleles at the 6 QTL for leaf rust resistance indicated that they could reduce the LR score by 1.70, whereas the 'Oberkulmer' alleles at the other 2 QTL could reduce the LR score by 0.55. Accordingly, the 'Forno' alleles at 6 QTL for leaf tip necrosis could increase LTN by 3.2 cm, whereas the 'Oberkulmer' alleles at the other 4 QTL could increase LTN by 1.62 cm.

The percentage of phenotypic variance explained by a single QTL (R^2) ranged from 7.2% to 35.8% for LR and from 6.8% to 18.0% for LTN. The QTL which explained most of the phenotypic variation for LR was on the long arm of chromosome 7B in the interval of 88–102 cM and


1A
1BS
1DS
2A
2B
2D
3A
3B
3DS
3DL
4A
4B
4DL
5A
5B
5DL
6A
6B
6D
7A
7B
7D
7S
0 cM
20 cM
40 cM
60 cM
80 cM
100 cM
QTL
R2
Leaf rust resistance: 8 51%
Leaf tip necrosis: 10 41%
R2 of QTL :
5 - 9 %
10 - 14%
15 - 20%
> 35%

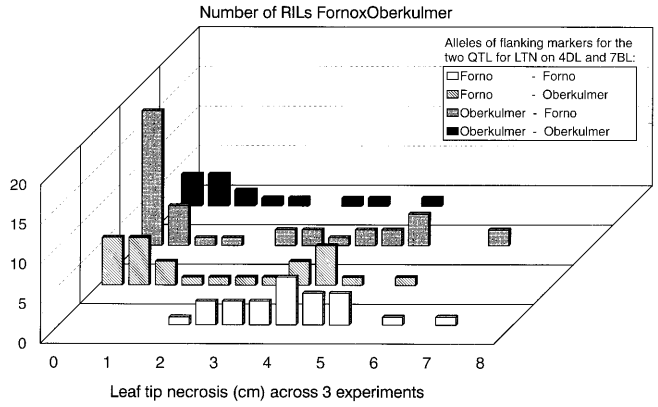


Fig. 3 Phenotypic distribution of the RILs of the cross 'Forno×Oberkulmer' divided into marker classes on the basis of their parental alleles at the marker interval *Xpsr1101a* and *Xpsr160b* on chromosome 4DL and at the marker interval *Xglk750* and *Xmwg710a* on chromosome 7BL, each containing a putative QTL for leaf tip necrosis

did not overlap with the support interval of the 2 QTL for LTN on 7B. At this QTL, one allele of 'Forno' contributed to a score reduction of 0.47; i.e., the homozygous classes of 'Forno' and 'Oberkulmer' alleles differed in a score of almost 1 for LR. The QTL with the greatest additive effect for LTN (–0.99 cm) was found on the same chromosome but more telomeric (140–162 cM), and it also had an effect on LR (+0.34 score); i.e., RILs homozygous for the 'Forno' allele showed on average 2 cm more LTN and 0.7 lower LR scores than those homozygous for the 'Oberkulmer' allele. Another predominant QTL for LTN (part. R^2=10.5%) was detected on 4DL (100–114 cM), which also coincided with a QTL for leaf rust resistance (+0.27 LR score). Both QTL for LTN performed in an additive manner. All of the RILs of 'Forno×Oberkulmer' that carry 'Forno' alleles at the closest marker loci for these QTL for LTN (4D, 110 cM and 7BL, 150 cM) clearly showed leaf tip necrosis of at least 2 cm (Fig. 3, 'Forno'-'Forno'), whereas those RILs that were homozygous for the 'Oberkulmer' alleles for at least 1 QTL locus showed a range of 0 (no leaf tip necrosis) to 7 cm for LTN. The RILs of the marker class 'Forno'-'Forno' had a mean of 4.0 cm for LTN, whereas the RILs of the marker class 'Oberkulmer'-'Oberkulmer' had on average only 1.2 cm LTN, with an average LR score of 2.3 and 3.7, respectively. The other two marker classes 'Forno'-'Oberkulmer' and 'Oberkulmer'-'Forno' showed intermediate values for LTN (1.9 and 1.8 cm) and for LR (3.8 and 3.1, respectively).

◀ **Fig. 2** Positions of significant (LOD>3.0) QTL for leaf rust resistance and leaf tip necrosis on the genetic map of 204 RILs derived from the cross 'Forno×Oberkulmer'. QTL for leaf rust resistance are indicated by triangles to the *right* of the chromosome and QTL for leaf tip necrosis by *triangles* to the *left* of the chromosome. The *size* of the *triangle* indicates the explained phenotypic variance (R^2) of a single QTL. *White* and *black triangles* indicate that the allele for improved leaf rust resistance or increased leaf-tip necrosis was inherited by the parent 'Forno' and 'Oberkulmer', respectively

All QTL detected across environments for LR were also significant in at least one of the four experiments, whereas 4 QTL for LTN on 3A, 6A, 7B (66–82 cM), and 7DS were significant across the three environments but not in the analysis of single environments. Significant (P<0.01) QTL×environment interactions were revealed for the putative QTL for LR on chromosomes 3A (58–66 cM) and 4DL (100–114 cM). The variance component of QTL×environment (0.03) was about 1/20 of the variance component explained by the QTL (0.63) and even smaller than the ratio between the genotype×environment interaction variance component ($\sigma^2_{G\times E}$=0.18) and the genotypic variance component (σ^2_G=1.32) obtained by the ANOVA of the phenotypic data.

Epistatic effects between QTL for LR and LTN

For leaf rust resistance we found significant (P<0.01) digenic epistatic effects between the putative QTL on chromosomes 1BS (30–34 cM) and 4B (32–54 cM) as well as between the QTL on 4B (32–54 cM) and 5DL (0–38 cM). If we include the digenic effects in the model for the simultaneous fit, the amount of explained phenotypic variance could be increased fom 50.9% to 54.0%, with a partial R^2 for the epistatic effects of 2.5 and 3.2%, respectively. The influence of QTL×QTL interaction on the phenotypic expression of leaf rust resistance is demonstrated for the QTL on 1BS and 4B. The RILs were divided into four classes based on the allele constitution of the closest marker loci for the 2 QTL. RILs that were homozygous for the 'Oberkulmer' allele at both QTL were on average less resistant (LR=3.7), whereas RILs that were homozygous for the 'Forno' alleles at the QTL on 1BS (LR=2.8) or at the QTL on 4B (LR=2.9) showed a similar resistance as the RILs homozygous for the 'Forno' alleles at both QTL (LR=2.9), indicating duplicate interaction between these QTL.

Digenic epistatic effects were also found between the putative QTL for LTN on chromosomes 3A (58–66 cM) and 7B (66–82 cM) (part. R^2=1.6%) and between the 2 QTL on 7B (66–82 cM) and 7B (140–162 cM) (part. R^2=2.3%). The additive effects of the 10 QTL for LTN together with their epistatic effects could explain 42.9% of the phenotypic variance in the simultaneous fit compared to 40.8% without epistatic effects.

Discussion

Genetic basis of durable leaf rust resistance

The objective of our study was to elucidate the genetic basis of the quantitative resistance of the Swiss winter wheat variety 'Forno'. The leaf rust resistance of 'Forno' has been shown to be durable over a period of 10 years of cultivation in Switzerland and, therefore, it is of great interest to transfer this resistance into other breeding lines. We found 8 genomic regions that were relevant for

the expression of leaf rust resistance under field conditions. 'Forno' alleles improve resistance at 6 of these QTL. Thus, the durable resistance of 'Forno' is oligogenic. The detected QTL represent a minimum number considering that we cover only about two-thirds of the genome with our genetic map (Messmer et al. 1999). The number of QTL for quantitative leaf rust resistance found in our study is higher than the number of genes estimated by segregation analysis (Shaner et al. 1997; VanderGaag and Jacobs 1997; Geiger and Heun 1989) under the assumption of equal gene effects. According to our data, this assumption is not met, since the additive effects of individual QTL for LR differed considerably (0.16–0.47). In a cross between the spring wheat 'Parula' and 'Siete Cerros', William et al. (1997) identified 2 genomic regions (1BS or 1DS and 7BL) conferring slow rusting using 400 RAPD markers. Nelson et al. (1997) detected 2 significant QTL on 7BL and 7DS for adult plant resistance in the field in a cross between synthetic wheat and the spring wheat 'Opata'. In both of these studies one of the parents contained the *Lr34* gene as well as other race-specific leaf rust resistance genes. Therefore, it is possible that the effect of minor genes was masked by these genes with major effects. Interestingly, in both studies a QTL was detected on chromosome 7BL, where we found the QTL with the largest effect in our population for each environment. Besides the 8 QTL for LR found across environments, 5 additional QTL (4 with 'Forno' alleles as the positive alleles) were detected in just one of the four environments. Since single environments differed in the time of plant development when leaf rust infection started, in the infection pressure, and most likely in the pathogen population, different genes might be relevant for resistance in different environments. Surprisingly, the flanking marker loci of 3 QTL that were found in only one environment and not over environments were revealed by the same probes as the flanking marker loci of QTL detected over environments: *Xpsr593a* on 1BS and *Xpsr593c* on 7B, *Xpsr1101b* on 3B and *Xpsr1101a* on 4DL and *Xpsr580b* on 5B and *Xpsr580a* on 5DL. It is possible that these QTL for leaf rust were duplicated or homoeologous loci. McMullen and Simcox (1995) found 3 regions on maize chromosomes 3, 5, and 8 with duplicated arrays of colinear RFLP loci, each containing a QTL for resistance to Northern corn leaf blight, which might represent duplications of the same gene or were derived from an ancient gene cluster.

In our study both parents contributed positive alleles for leaf rust resistance, thereby allowing transgression breeding. Based on the QTL results and the fact that the mean of the F_5 RILs was not significantly different from the parental mean, additive effects were the predominant mode of inheritance for leaf rust resistance. However, we found significant epistatic effects between 1 QTL on 4B and the QTL on 1BS and 5DL for improved field resistance. This is in agreement with Das et al. (1992), who found predominantly additive genetic variance for partial leaf rust resistance in advanced spring wheat populations as well as additive×additive genetic variance. Since the F_1 hybrid of our cross 'Forno×Oberkulmer' showed partial dominance for leaf rust resistance in field trials, dominance variance might also play a role in the inheritance of durable leaf rust resistance. Consequently, the selection for durable leaf rust resistance in early generations might be less effective than in later generations.

Genetic basis of leaf tip necrosis

Since leaf tip necrosis has been reported to be a monogenic trait (*Ltn*) located on 7DS (Singh 1992; William et al. 1997), we first started to assess it as a qualitative trait. However, the results were not reproducible and depended on the time of the assessment. As the extent of leaf tip necrosis varied continuously between genotypes, we made a quantitative assessment. Afterwards, the genotypes were divided into two distinct classes according to the bimodal distribution (A ≥2 cm LTN, 0 cm ≤B ≤1 cm LTN) and LTN was mapped as a monogenic trait. Since we found no significant linkage to any marker loci on 7D nor to any other of the 230 marker loci of the genetic map, we concluded that the gene for LTN of 'Forno' is either not located on 7D but on chromosomal regions not covered by marker loci or that LTN of 'Forno' is not a monogenic trait. Although we found a QTL for the quantitative assessment of LTN on 7DS (part. R^2=5%) where the *Ltn* gene was mapped (Singh 1992), the QTL explaining most of the phenotypic variation were on 7BL (part. R^2=13%), 4DL (part. R^2=11%), and 5A (part. R^2=9%). The distribution of the 'Forno×Oberkulmer' population for LTN at the 2 major QTL revealed that at least 2 genes with additive gene action are responsible for the expression of leaf tip necrosis in the 'Forno× Oberkulmer' population: all RILs with 'Forno' alleles at both QTL on 7BL and 4DL showed leaf tip necrosis (2–7cm). Therefore, the genetic basis of leaf tip necrosis observed in the Swiss winter wheat 'Forno' is different from the one reported for CIMMYT spring wheat (Singh 1992).

Pleiotropic effects of leaf rust resistance and leaf tip necrosis

In the CIMMYT spring wheat material leaf tip necrosis can be used as morphological marker for the presence of the durable resistance gene *Lr34* (Singh 1992). Although different genes are involved in the expression of leaf tip necrosis in 'Forno', 32% of the phenotypic variation of leaf rust resistance could be explained by LTN. Since half of the QTL for adult plant leaf rust resistance coincided with QTL for LTN, the association of leaf rust resistance with leaf tip necrosis is more likely due to pleiotropic effects than the close linkage of LR and LTN genes on four different chromosomes. Singh and Huerta-Espino (1997) hypothesized that resistance conferred by *Lr34* may involve the production of toxic metabolites,

which may in turn induce leaf tip necrosis. Vice versa, leaf tip necrosis may change the physiology of the flag leaf, which could disturb the infection process and the growth of the pathogen. Hu and Rijkenberg (1998) studied the infection process of *Puccinia recondita* in wheat and argued that physical and chemical features of the leaf surface like cuticular ridges, patterns of epicuticular wax crystals, or pH gradients of the leaf surface might affect the direction of pathogen growth.

Different physiological states (earlier senescence) of the flag leaf might be less attractive or prosperous to the pathogen and might explain the occurrence of associated resistance genes against various pathogens. The adult plant leaf rust resistance gene *Lr34*, which is closely linked or pleiotropic to *Ltn*, is also associated with the stripe rust resistance gene *Yr18* and the *bdv1* resistance gene against the barley yellow dwarf virus (Singh 1993). In our population, 4 of the 8 QTL for leaf rust resistance (3A, 4DL, 7BL, 7BL) coincided with 4 of the 18 QTL found for powdery mildew resistance (Keller et al. 1999a), and 3 of these genomic regions were also involved in the expression of LTN. Coincidences of QTL against different diseases have also been reported for maize (McMullen and Simcox 1995). However, it will be very difficult to determine if the resistance against various diseases are due to the pleiotropic effects of 1 gene or due to clusters of genes involved in plant defense (Spielmeyer et al. 1998; Li et al. 1999).

Thaumatin as a candidate gene for a QTL for leaf rust resistance

One QTL for LR on 7BL (92 cM) had a large effect on leaf rust resistance in each environment, explaining one-quarter of the phenotypic variation. Therefore, we assume that this QTL represents a major gene for adult plant resistance. Strikingly, the support interval of this QTL includes the *pwir232b* locus located 1 cM apart from the most likely QTL position. This cDNA clone was isolated from wheat infected by barley powdery mildew (*Erysiphe graminis*) and encodes a pathogen-induced thaumatin-like protein (Rebmann et al. 1991). It belongs to the pathogenesis-related (PR)-5 proteins that are induced by different phytopathogens in many plant species and characterized by sequence similarity with thaumatin (Lin et al. 1996; Hu and Reddy 1997). Purified PR-5 proteins from *Arabidopsis* (Hu and Reddy 1997), maize and other cereals (Lin et al. 1996) showed antifungal activity. Therefore, *pwir232b* is a candidate gene for leaf rust resistance. In the same genomic region on 7BL we found as well a QTL for powdery mildew resistance explaining 11% of the phenotypic variance (Keller et al. 1999a). Recently, Li et al. (1999) located 62 candidate genes for defense response on the comprehensive genetic map of the synthetic wheat×'Opata' cross, which was used to identify quantitative disease resistance genes (Nelson et al. 1997; Faris et al. 1999). Since Faris et al. (1999) also found a strong association between a cluster of defense resistance genes (catalase, thaumatin, chitinase, ion channel regulator genes) on 7BL and resistance against leaf rust under natural infection pressure, Karnal bunt, and stem rust, the same candidate gene might be active in very different wheat lines against various diseases. Whether the thaumatin gene of 'Forno' or one of the other defence genes is responsible for the improved leaf rust resistance can only be proven by transformation experiments.

Coincidence of QTL for leaf rust resistance with major *Lr* genes

Robertson (1989) postulated that qualitative mutant phenotypes are extreme alleles at a QTL. Given this hypothesis, we compared the genomic regions involved in the quantitative expression of leaf rust resistance with the map location of race-specific *Lr* genes (McIntosh et al. 1998). The QTL on chromosome 1B could correspond to seedling resistance genes *Lr2a,b,c*, *Lr26a* (1B/1R translocation) or *Lr44* (*T. spelta*; Dyck and Sykes 1994) or the adult-plant gene *Lr46* (Singh et al. 1998). The QTL on 2BS is about 15 cM proximal to the centromere and, therefore, could be allelic to the adult plant resistance gene *Lr13* mapped by Seyfarth et al. (1998). On the same genomic region Nelson et al. (1995a,b) found a QTL for leaf rust resistance which together with *Lr34* explained 45% of the phenotypic variation. *Lr23* is also close to the centromere on 2BS (Hart et al. 1993; Nelson et al. 1997). Several *Lr* genes are mapped on 4BS (*Lr25*, *Lr31*) or located on chromosome 4B (*Lr16*, and adult plant resistance gene *Lr12*) (McIntosh et al. 1998). *Lr1* was mapped close to the telomere of 5DL (Feuillet et al. 1995) and, therefore, does not overlap with the QTL interval on 5D (0–38 cM). The QTL on 7BL (150 cM) might correspond to the *Lr14a* locus which was mapped 12 cM distal to the *Pm5* gene (Hart et al. 1993). No *Lr* genes are mapped on chromosome 3A and 4DL. However, more work is needed to verify if the observed coincidence of QTL and major genes is due to allelism or due to close linkage.

Because of the occurrence of leaf tip necrosis in the durable leaf rust resistant wheat parent 'Forno' we expected to find a QTL resembling the *Lr34* adult plant resistance gene. Since we could not detect a significant QTL on 7DS, but only several genomic regions associated with LR and LTN, it is possible that 'Forno' carries one or more homoeologous loci of *Lr34* carrying different alleles (e.g., PRS160, a flanking marker of the QTL on 4DL, also hybridized to 7DS). Similarly, Dyck et al. (1994) proved that two lines with the phenotype of *Lr34* (improved leaf rust resistance associated with adult plant stripe resistance and leaf tip necrosis) might have the same gene or gene complex but at different chromosomal locations. Based on the presence of quadrivalents in pollen mother cells they concluded that *Lr34* might be translocated onto another chromosome in their wheat line.

The QTL analysis allowed the elucidation of inheritance of the durable leaf rust resistance of the Swiss winter wheat variety 'Forno' and the genetic association between leaf rust resistance and leaf tip necrosis. At least 6 genes are responsible for the high level of resistance in 'Forno', 4 of them are pleiotropic or closely linked with leaf tip necrosis. Additive effects are of major importance, while epistatic effects seem to be of minor importance. Dominance effects for leaf rust resistance might mask the genotypic value in early generations. Because of the oligogenic inheritance it is rather difficult to introgress the high level of resistance from 'Forno' into other breeding material by phenotypic selection. Although we could not identify the presence of the durable leaf rust resistance gene *Lr34*, phenotypic selection for leaf tip necrosis would improve the level of resistance in the breeding material. However, breeders often select against leaf tip necrosis because varieties with strong leaf tip necrosis, like 'Forno', are not well accepted by the farmers. With marker-assisted selection for the QTL on 7B (92 cM) and 1BS (32 cM) the level of leaf rust could be improved considerably without selection for leaf tip necrosis. Moreover, QTL for partial resistance can be combined with race-specific *Lr* genes by marker assisted selection in order to breed varieties with a high level of resistance that is effective over a long period of time.

Acknowledgements We thank S. Hitz, Ph. Streckeisen, E. Sager and S. Boller for their excellent technical assistance and Dr. R. Dudler (University of Zürich, Switzerland) for providing the PWIR clone. We are grateful to Prof. Dr. H.F. Utz for providing the computer packages PLABSTAT and PLABQTL. This work was supported by the Swiss Priority Program Biotechnology (Project Numbers: 5002–38967 and 5002–045033). The authors declare that the experiments conducted for this publication comply with the current laws of Switzerland.

References

Bohn M, Khairallah M, Gonzales-de-Leon D, Hoisington A, Utz HF, Deutsch JA, Jewell DC, Mihm JA, Melchinger AE. (1996) QTL mapping in tropical maize: I. genomic regions affecting leaf feeding resistance to sugar cane borer and other traits. Crop Sci 36:1352–1361

Das MK, Rajaram S, Mundt CC, Kronstad WE (1992) Inheritance of slow-rusting resistance in wheat. Crop Sci 32:1452–1456

Dyck PL, Sykes EE (1994) Genetics of leaf-rust resistance in three spelt wheats. Can J Plant Sci 74:231–233

Dyck PL, Kerber ER, Aung T (1994) An interchromosomal reciprocal translocation on wheat involving leaf rust resistance gene *Lr34*. Genome 37:556–559

Faris JD, Li WL, Liu DJ, Chen PD, Gill BS (1999) Candidate gene analysis of quantitative disease resistance genes. Theor Appl Genet 98:219–225

Feuillet C, Messmer M, Schachermayr G, Keller B (1995) Genetic and physical characterization of the *Lr1* leaf rust resistance locus in wheat (*Triticum aestivum* L.) Mol Gen Genet 248:553–562

Geiger HH, Heun M (1989) Genetics of quantitative resistance to fungal diseases. Annu Rev Phytopathol 27:317–341

Hallauer AR, Miranda Fo JB (1981) Quantitative genetics in maize breeding. The Iowa University Press, Ames

Hart GE, Gale MD, McIntosh RA (1993) Linkage maps of *Triticum aestivum* (hexaploid wheat, 2n=42, genomes A, B, and D) and *T. tauschii* (2n=14, genome D). In: O'Brien SJ (ed) Genetic maps: locus maps of complex genomes. Cold Spring Harbor Laboratory Press, pp 6.204–6.219

Hu X, Reddy ASN (1997) Cloning and expression of a PR5-like protein from *Arabidopsis*: inhibition of fungal growth by bacterially expressed protein. Plant Mol Biol 34:949–959

Hu G, Rijkenberg FHJ (1998) Scanning electron microscopy of early infection structure formation by *Puccinia recondita* f. sp. *tritici* on and in susceptible and resistant wheat lines. Mycol Res 102:391–399

Johnson R (1983) Genetic background of durable resistances. In: Lamberti F, Waler JM, Van der Graaf NA (eds) Durable resistance in crops. Plenum Press, New York, pp 5–26

Keller M, Keller B, Schachermayr G, Winzeler M, Schmid JE, Stamp P, Messmer MM (1999a) Quantitative trait loci for resistance against powdery mildew in a segregating wheat×spelt cross. Theor Appl Genet 98:903–912

Keller M, Karutz Ch, Schmid JE, Stamp P, Winzeler M, Keller B, Messmer MM (1999b) Quantitative trait loci for lodging resistance in a segregating wheat×spelt cross. Theor Appl Genet 98:1171–1182

Kolmer JA (1996) Genetics of resistance to wheat leaf rust. Annu Rev Phytopathol 34:435–455

Li WL, Faris JD, Chittoor JM, Leach JE, Hulbert SH, Liu DJ, Chen PD, Gill BS (1999) Genomic mapping of defense response genes in wheat. Theor Appl Genet 98:226–233

Lin KC, Bushnell WR, Szabo LJ, Smith AG (1996) Isolation and expression of a host response gene family encoding thaumatin–like proteins in incompatible oat-stem rust fungus interactions. Mol Plant-Microbe Inter 9:511–522

McIntosh RA, Wellings CR, Park RF (1995) Wheat rust: an atlas of resistance genes. CSIRO Australia and Kluwer Academic Publ, Dordrecht, The Netherlands

McIntosh RA, Hart GE, Devos KM, Gale MD, Rogers WJ (1998) Catalogue of gene symbols for wheat. In: Slinkard AE (ed) Proc 9th Int Wheat Genet Symp, Vol 5. University Extension Press, Saskatoon, Saskatchewan, Canada, pp134–139

McMullen MD, Simcox KD (1995) Genomic organization of disease and insect resistance genes in maize. Mol Plant-Microbe Inter 8:811–815

Messmer MM, Keller M, Zanetti S, Keller B (1999) Genetic linkage map of a wheat×spelt cross. Theor Appl Genet 98:1163–1170

Nelson JC, Van Deynze AE, Autrique E, Sorrells ME, Lu YH, Merlino M, Atkinson M, Leroy P (1995a) Molecular mapping of wheat homoeologous group 2. Genome 38:516–524

Nelson JC, Sorrells ME, Van Deynze AE, Lu YH, Atkinson M, Bernard M, Leroy P, Faris JD, Anderson JA (1995b) Molecular mapping of wheat. Major genes and rearrangements in homoeologous groups 4, 5, and 7. Genetics 141:721–731

Nelson JC, Singh RP, Autrique JE, Sorrells ME (1997) Mapping genes conferring and suppressing leaf rust resistance in wheat. Crop Sci 37:1928–1935

Rebmann G, Mauch F, Dudler R (1991) Sequence of a wheat cDNA encoding a pathogen-induced thaumatin-like protein. Plant Mol Biol 17:283–285

Robertson DS (1989) Understanding the relationship between qualitative and quantitative genetics. In: Helentjaris T, Burr B (eds) Development of application of molecular markers to problems in plant genetics. Cold Spring Harbor Laboratory Press, New York, pp 8187

Seyfarth R, Feuillet C, Keller B (1998) Development and characterization of molecular markers for the adult plant leaf rust resistance genes *Lr13* and *Lr35* in wheat. In: Slinkard AE (ed) Proc 9th Int Wheat Genet Symp Vol 3. University Extension Press, Saskatoon, Saskatchewan, Canada, p 154

Shaner G, Buechley G, Nyquist WE (1997) Inheritance of latent period of *Puccinia recondita* in wheat. Crop Sci 37:748–756

Singh RP (1992) Association between gene *Lr34* for leaf rust resistance and leaf tip necrosis in wheat. Crop Sci 32:874–878

Singh RP (1993) Genetic association of gene *Bdv1* for tolerance to barley yellow dwarf virus with genes *Lr34* and *Yr18* for adult plant resistance to rusts in bread wheat. Plant Dis 77:1103–1106

Singh RP, Rajaram S (1992) Genetics of adult-plant resistance of leaf rust in 'Frontana' andthree CIMMYT wheats. Genome 35:24–31

Singh RP, Huerta-Espino J (1997) Effect of leaf rust resistance gene *Lr34* on grain yield and agronomic traits of spring wheat. Crop Sci 37: 390–395

Singh RP, Mujeeb-Kazi A, Huerta-Espino J (1998) *Lr46*: a gene conferring slow-rusting resistance to leaf rust in wheat. Phytopathology 88:890–894

Spielmeyer W, Robertson M, Collins N, Leister D, Schulze-Lefert P, Seah S, Moullet O, Lagudah ES (1998) A superfamily of disease resistance gene analogs is located on all homoeologous chromosome groups of wheat (*Triticum aestivum*). Genome 41:782–788

Utz HF (1995) PLABSTAT – Ein Computerprogramm zur statistischen Analyse von pflanzenzüchterischen Experimenten. Version 2 M. Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Stuttgart, Germany

Utz HF, Melchinger AE (1996) PLABQTL. A computer program to map QTL. Version 1.1, Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Stuttgart, Germany

VanderGaag DJ, Jacobs T (1997) Inheritance of host plant effect on latent period of wheat leaf rust in single-seed descent F_8 lines. Euphytica 93:67–72

William HM, Hoisington D, Singh RP, Gonzalez-de-Leon D (1997) Detection of quantitative trait loci associated with leaf rust resistance in bread wheat. Genome 40:253–260

Winzeler M, Winzeler H, Keller B, Streckeisen Ph, Hitz S, Messmer M, Schachermayr G, Feuillet C (1995) Strategien der Züchtung auf Braunrostresistenz bei Weizen. 46. Arbeitstagung der Saatzuchtleiter im Rahmen der "Vereinigung österreichischer Pflanzenzüchter". BAL, Gumpenstein, pp 83–88

Zadoks JC, Chang TT, Konzak CF (1974) A decimal code for the growth stages of cereals . Weed Res 14:415–421