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ORIGINAL PAPER

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Dissection of quantitative and durable leaf rust resistance in Swiss winter wheat reveals a major resistance QTL in the *Lr34* chromosomal region

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Abstract The Swiss winter bread wheat *cv.* 'Forno' has a highly effective, durable and quantitative leaf rust (*Puccinia triticina* Eriks.) resistance which is associated with leaf tip necrosis (LTN). We studied 240 single seed descent lines of an 'Arina×Forno' $F_{5:7}$ population to identify and map quantitative trait loci (QTLs) for leaf rust resistance and LTN. Percentage of infected leaf area (%) and the response to infection (RI) were evaluated in seven field trials and were transformed to the area under the disease progress curves (AUDPC). Using composite interval mapping and LOD >4.4, we identified eight chromosomal regions specifically associated with resistance. The largest and most consistent leaf rust resistance locus was identified on the short arm of chromosome 7D (32.6% of variance explained for AUDPC_% and 42.6% for AUDPC_RI) together with the major QTL for LTN (R^2=55.6%) in the same chromosomal region as *Lr34* (*Xgwm295*). A second major leaf rust resistance QTL (R^2=28% and 31.5%, respectively) was located on chromosome arm 1BS close to *Xgwm604* and was not associated with LTN. Additional minor QTLs for LTN (2DL, 3DL, 4BS and 5AL) and leaf rust resistance were identified. These latter QTLs might correspond to the leaf rust resistance genes *Lr2* or *Lr22* (2DS) and *Lr14a* (7BL).

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Introduction

The wheat leaf rust fungus (*Puccinia triticina* Eriks.) is endemic in all wheat (*Triticum aestivum* L.) growing areas worldwide. Susceptible varieties are frequently severely infected and breeding for resistance is environmentally and economically the most appropriate strategy to fight this disease sustainably. In Switzerland, wheat is naturally infested each year and durable resistance against leaf rust is highly valuable.

The introgression and pyramiding of various leaf rust resistance (*Lr*) genes was the first attempt to use the present genetic diversity within hexaploid wheat and its wild relatives against this disease (McIntosh et al. 1995b). Monogenetically inherited *Lr* resistance genes have usually been rapidly overcome by rapidly evolving pathotypes of the fungus (McIntosh et al. 1995b; Kolmer 1996). Of the almost 50 known *Lr* resistance genes, two (*Lr34* and *Lr46*) can be classified as slow-rusting genes (van der Plank 1963; Parlevliet 1979; Singh and Gupta 1991; Singh et al. 1998). In contrast to race-specific resistance genes, *Lr34* and *Lr46* have proved to confer resistance over a long period of time, in different environments and against diverse pathotypes of the fungus (Singh and Rajaram 1992; Singh et al. 1998). Genetic studies using lines from the CIMMYT (Nelson et al. 1997; William et al. 1997), the Japanese and Israeli (Suenaga et al. 2003) or the Indian (Kaur et al. 2000) spring wheat germplasm confirmed the involvement of both genes in the expression of durable leaf rust resistance. Not much is known about the occurrence of these two genes within the European winter wheat germplasm. In an interspecific cross between the Swiss winter bread wheat (*T. aestivum* L.) *cv.* 'Forno' and a spelt (*T. spelta* L.), *cv.* 'Oberkulmer', it was shown that

the quantitative and durable leaf rust resistance expressed in *cv.* 'Forno' is also based on a partially resistant or slow-rusting phenotype (Messmer et al. 2000). In addition, during the adult plant stage 'Forno' develops leaf tip necrosis (LTN) on the flag leaf.

From the CIMMYT spring wheat germplasm, it is known that the *Ltn* gene conferring LTN is associated with the slow-rusting and durable adult plant resistance gene *Lr34* (Singh 1992a) which was mapped to chromosome arm 7DS close to *Xgwm295* and *Xwg834* (Nelson et al. 1997; Suenaga et al. 2003). It is still not clear, however, whether *Ltn* is pleiotropic to or tightly linked with *Lr34*. In addition, the *Ltn*/*Lr34* chromosomal segment also confers a durable resistance to stripe (yellow) rust, *Yr18* (Singh 1992b), stem rust (Liu and Kolmer 1998), as well as tolerance against the barley yellow dwarf virus, *Bdv1* (Singh 1993; Ayala et al. 2002) and inactivates a stem rust resistance suppressor (Kerber and Aung 1999). Thus, *Ltn*/*Lr34* is unique and a very valuable source of disease resistance in wheat breeding.

During the seedling stage, wheat lines with *Lr34* show a susceptible infection type (Rubiales and Niks 1995). In the adult plant stage, the *Lr34* phenotype typically exhibits a rust pustule gradient on the flag leaf with more and larger pustules at the leaf base and fewer and smaller pustules towards the leaf tip. Usually, the pustule gradient goes along with the presence of *Ltn* (Singh 1992a; Kolmer 1996). Histological studies investigating the partial resistance mechanism expressed in 'Tc-*Lr34*' (*Lr34* introgressed into the susceptible genetic background of *cv.* 'Thatcher') revealed that the resistance was due to neither hypersensitivity nor to penetration resistance based on cell wall appositions (papillae) (Rubiales and Niks 1995). In fact, the partial resistance in 'Tc-*Lr34*' was significantly and mainly based on early abortion events of the leaf rust germ tubes which failed to enter leaf mesophyll cells as well as delayed hyphal growth and smaller colony size during the adult plant stage (Rubiales and Niks 1995; Kloppers and Pretorius 1997).

Several leaf rust resistance studies described an enhanced resistance effect when *Lr34* was combined with one or more adult plant resistance (APR) or seedling resistance genes such as *Lr2*, *Lr12*, *Lr13* or *Lr16*, which give a hypersensitive response (HR). In addition, the durable and slow rusting type of resistance remained effective even when the resistance of the major *Lr* resistance gene(s) was already ineffective (German and Kolmer 1992; Sawhney 1992; Kolmer 1996; Kloppers and Pretorius 1997).

The objective of this study was to dissect the quantitative and durable leaf rust resistance of the Swiss *cv.* 'Forno' in the 'Arina×Forno' population using quantitative trait locus (QTL) analysis.

Materials and methods

Plant material

Two hundred and forty single seed descent lines from the intraspecific cross between the two adapted Swiss winter bread wheat cultivars 'Arina' and 'Forno' were generated ($F_{5:7}$) and phenotyped in field trials for their leaf rust resistance. The pedigrees of both parents are as follows (Martynov et al. 1992):

- 'Arina'=Moisson/Zenith
- 'Forno'=NR-72-837/Kormoran
- 'Kormoran'=Cappelle Desprez/Heine-2806//Heine-646
- 'Moisson'=80-30-Versailles/Etoile de Choisy//Cappelle Desprez
- 'Zenith'=Can3842/Heine VII

'Forno' was released in 1986 and covered up to 14% of the wheat area in Switzerland until 1997. It maintained a high level of resistance against leaf rust. Additionally, broad effectiveness of the leaf rust resistance expressed in *cv.* 'Forno' was confirmed in tests of a European wheat leaf rust resistance survey, where it was exposed to various environments and pathotypes across Europe (Winzeler et al. 2000). It can therefore be assumed that this resistance is durable. In contrast, *cv.* 'Arina' is highly susceptible to leaf rust.

Field experiments

Seven field trials were conducted in 1999 and 2000 at different locations representing the diverse wheat growing areas in Switzerland (Schnurbusch et al. 2003). One field trial was located in Vouvry (Vouvry00), two were in Haag (Haag99, Haag00), and four in Zürich-Reckenholz (Flü99, Grü99, ZH106, and ZH114). The lines were grown as described in Schnurbusch et al. (2003).

Field infection, disease phenotyping and trait assessment

All field trials were artificially inoculated with a mixture of 16 prevalent Swiss leaf rust isolates as described in Messmer et al. (2000). All standard cultivars, the reciprocal F_1, the parental lines and the lines of the 'Arina×Forno' population were phenotyped on the flag leaf in seven environments by visually estimating the percentage of infected leaf area according to the modified Cobb scale (Peterson et al. 1948), and response to infection (RI, except Vouvry00 where only the third scoring for RI was possible). The RI considers the pustule size as well as the necrotic leaf area and was scored as follows:

- 5=S=susceptible (large pustules with no necrosis and little or no chlorosis present)
- 4=MS=moderately susceptible (medium sized pustules with no necrosis, possible some distinct chlorosis)
- 3=MR=moderately resistant (small pustules present surrounded by necrotic halos)
- 2=R=resistant (necrotic areas with or without minute pustules)
- 1=zero=no visible symptoms

In all tested environments, two replications were evaluated three times, every 6–7 days. The first scoring started when the flag leaf of the susceptible parent 'Arina' had 10–15% of infected leaf area. Plant height (cm; in six environments) and heading time (days after 1 January; in five environments) as well as LTN (mm; in all environments, except ZH114), glaucousness (waxy versus non-waxy flag leaf; in four environments) and flag leaf status (erect versus droopy flag leaf; in four environments) were determined in two replications for the 'Arina×Forno' population. Glaucousness (area of waxy bloom on the lower side of the flag leaf) and flag leaf status were evaluated once during heading time on a scale from one to five and one to four, respectively. For the scoring of both

characters, we used for glaucousness a 1 (=non-waxy) to 5 (=waxy flag leaf) scale, and for leaf status a 1 (=droopy) to 4 (=erect flag leaf) scale. Once during anthesis, LTN was measured with a ruler on five flag leaves of each line along the leaf axis from the necrotic leaf tip to the beginning of the green leaf tissue.

Molecular markers

All 240 lines of the 'Arina×Forno' population were genotyped with molecular markers. A genetic linkage map based on microsatellite (single sequence repeat) and restriction fragment length polymorphism (RFLP) markers was established (Paillard et al. 2003). The linkage map spans 3,086 cM with 380 loci distributed into 27 linkage groups with an average marker density of around 8 cM (Paillard et al. 2003).

Statistical analysis

Phenotypic data

Lattice analysis of single environments and analysis of variance across environments were performed with the program PLABSTAT, version 2 M (Utz 1995), on all tested traits. The obtained adjusted entry means from single environments were used to compute the analysis of variance across environments. Components of variance were computed considering the effects of the environment and genotype as random. Estimates of variance components σ_G^2 (genetic variance), σ_E^2 (environment variance), σ_{GxE}^2 (genotype × environment interaction variance) and σ_{Err}^2 (error variance) were calculated. Broad-sense heritabilities were calculated on an entry mean basis according to Hallauer and Miranda Fo (1981). The distribution of the lines for leaf rust susceptibility was tested for normality using the SAS univariate procedure (SAS Institute 1991). Phenotypic correlation coefficients of leaf rust scores between the environments were calculated on an entry mean basis. The area under the disease progress curve (AUDPC) for AUDPC_% (infected leaf area) and AUDPC_RI (response to infection), respectively, was calculated based on three leaf rust scorings per environment (Campbell and Madden 1990). For the transformation of RI to AUDPC_RI we used the numbers 1–5. The adjusted entry means for the AUDPC_% per environment were used to estimate the genotypic values across seven environments. The estimation of the genotypic values for AUDPC_RI is based on adjusted entry means from six environments.

Marker data and QTL analysis

QTL analysis was performed for all single markers from the 'Arina×Forno' map by a simple one-way ANOVA using the SAS GLM procedure (SAS Institute 1991). Interval QTL analysis was carried out with the composite interval mapping (CIM) program PLABQTL, version 1.1 (Utz and Melchinger 2000), which is based on multiple regression. Twenty-five markers that were linked more closely than 0.2 cM were excluded from the QTL analysis to prevent ill-conditioned equation systems and the generation of "synthetic" new markers by the program. In order to determine the significance of a QTL for simple interval mapping (SIM) and CIM, the critical LOD (logarithm of the odds) thresholds were determined executing a permutation test for each trait with 1,000 permutations. For SIM and CIM, the critical LOD thresholds were set to LOD >2.5 and >4.4, respectively, because the individual critical LOD thresholds at a type-I error rate of α=0.25 (Beavis 1998) for AUDPC_%, AUDPC_RI and *Ltn* resulted in 2.38, 2.35 and 2.37 for SIM, respectively, and 4.34, 4.39 and 4.39 for CIM, respectively. After calculating SIM for each trait, a whole-genome scan with CIM was conducted using the automatic covariate selection statement ('cov select'). Selected covariates were checked individually for overly tight linkage or accumulation close to a detected QTL as recommended in the user's manual. In all interval mapping runs, we used the 'model AA' statement estimating only additive effects due to the low heterozygosity of our population. Detected epistatic effects (digenic QTL×QTL interactions) were added to the additive effects in the model.

Results

Phenotypic data for leaf rust resistance and LTN

The continuous phenotypic frequency distributions across all tested environments for both leaf rust scorings, AUDPC_% (Fig. 1A) and AUDPC_RI, differed significantly from normality (P<0.0001). Transgression for susceptibility and resistance occurred frequently within the 'Arina×Forno' population (Fig. 1A). AUDPC_% and AUDPC_RI were highly correlated (r=0.94, P<0.01) and highly heritable with 0.94 and 0.89, respectively (Table 1). More than 95% of the values for the RI within the population ranged from 'MR' to 'S' with most of the lines belonging to the 'MS' category (data not shown). Therefore, in our population, RI reflects pustule size and not resistance based on the HR. For the third leaf rust scoring, we found on average 10% of infected leaf area on the flag leaf of the resistant *cv.* 'Forno', whereas 'Arina' had 65% ($LSD_{5\%}$=9.5%; Table 2). Pustule sizes were also significantly ($LSD_{5\%}$=0.6) distinct from each other. The third leaf rust scoring of 'Forno' on the flag leaf was on average 'MS' (=4.26) and for 'Arina', 'S' (=5.0) (Table 2). In Vouvry00 we detected the highest average leaf rust infestation for the population as a whole and in Haag00 the lowest (Table 2). Some lines of the cross exhibited the specific *Lr34* phenotype (Kolmer 1996), expressing LTN and a pustule gradient (large pustules at the basis of the flag leaf and less and fewer pustules towards the leaf tip).

The average length for LTN was significantly ($LSD_{5\%}$=7.9 mm) different between both parents. It was 23.0 mm ±2.4 for the resistant parent 'Forno' and 5.7 mm ±0.7 for 'Arina' (Table 2). In Vouvry00, we measured the longest average LTN for the population and in ZH106 the shortest (Table 2). The bimodal and continuous phenotypic frequency distribution for LTN differed significantly from normality (P<0.0001), with transgression for longer LTN more frequent (Fig. 1B). Although we observed within-row (=within-line) variation, the heritability of LTN (0.86) based on five randomly measured flag leaves was still very high (Table 1).

Leaf rust susceptibility correlated (P<0.01) with LTN (r=–0.63 for AUDPC_% and r=–0.58 for AUDPC_RI, respectively) but not with any other leaf trait such as leaf status or glaucousness nor with any morphological character such as plant height or heading time (Table 1). Finally, highly susceptible lines exhibiting LTN were not found (Fig. 1C).

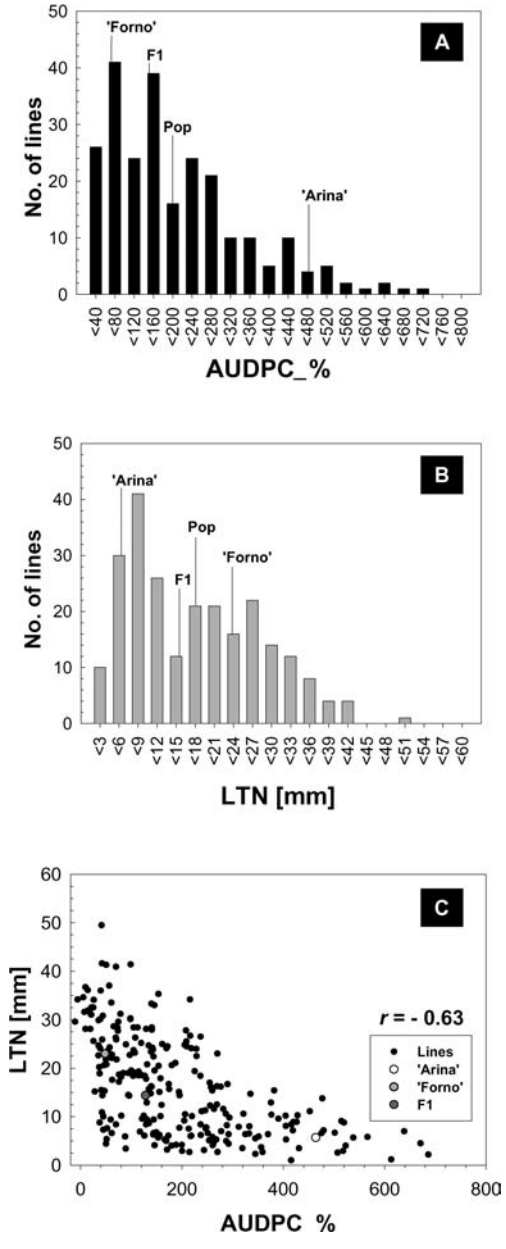

Fig. 1A–C Phenotypic data for leaf rust resistance and leaf tip necrosis across all tested environments for the 'Arina×Forno' population. **A** Phenotypic distribution for the area under the disease progress curve for the percentage of infected leaf area (AUDPC_%). **B** Phenotypic distribution of leaf tip necrosis (LTN). **C** Scatter plot of the phenotypic correlation between leaf rust susceptibility (AUDPC_%) and LTN (P<0.01; r=–0.63)

QTLs for leaf rust resistance, LTN and their interaction

Eight chromosomal regions conferring leaf rust resistance within the 'Arina×Forno' population were detected across all tested environments using CIM and a critical LOD of 4.4 (Table 3). Six resistance alleles originated from the resistant parent 'Forno' and the remaining two from the susceptible *cv.* 'Arina' (Table 3). One resistance locus on chromosome arm 7DS contributed to three traits (AUDPC_%, AUDPC_RI, LTN; Table 3, Fig. 2C). Three

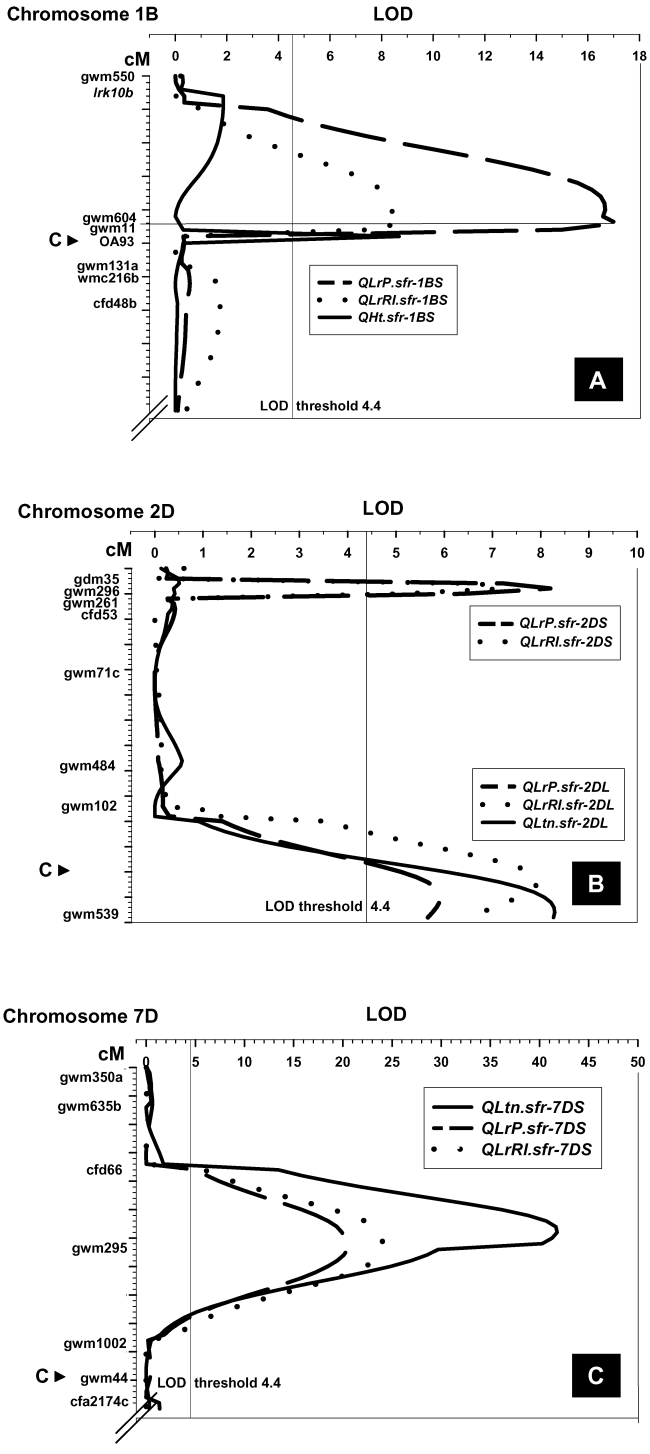

Fig. 2A–C Quantitative trait loci (QTLs) for leaf rust resistance and leaf tip necrosis (LTN) in the 'Arina×Forno' population. **A** Leaf rust resistance QTLs for the area under the disease progress curve (AUDPC) for the percentage of infected leaf area (AUDPC_%=*QLrP.sfr*) and the AUDPC for the response to infection (AUDPC_RI=*QLrRI.sfr*) on chromosome 1B short arm close to a plant height QTL, *QHt.sfr-1BS*. **B** Leaf rust resistance QTLs on the short and long arm of chromosome 2D as well as one QTL for LTN, *QLtn.sfr-2DL*. **C** Leaf rust resistance QTLs on the short arm of chromosome 7D as well as one QTL for LTN, *QLtn.sfr-7DS*. One dash on the *y*-axis represents 2 cM

Table 1 Phenotypic correlation coefficients between leaf rust susceptibility and other traits. Leaf rust susceptibility was determined as the area under the disease progress curve for the percentage of infected leaf area (AUDPC_%) and for the response to infection (AUDPC_RI). Broad-sense heritabilities and the number of environments in which various traits were evaluated are also listed

Trait	Phenotypic correlation coefficient		Heritability	No. of environments
	AUDPC_%	AUDPC_RI		
AUDPC_%	1	0.94*	0.94	7
AUDPC_RI	0.94*	1	0.89	6
LTN[a]	–0.63*	–0.58*	0.86	6
Heading time	–0.24	–0.25	0.92	5
Glaucousness[b]	0.09	0.14	0.96	4
Flag leaf status[c]	–0.07	0	0.91	4
Plant height	–0.07	–0.02	0.95	6

* $P<0.01$

[a] Leaf tip necrosis measured in millimetres (mm) on five randomly chosen flag leaves per row

[b] Glaucousness (area of waxy bloom on the lower side of the flag leaf)

[c] Flag leaf status was evaluated as erected versus droopy

Table 2 Single environment and average leaf rust score at the peak of the epidemic. Leaf rust scorings compared to the length of leaf tip necrosis (LTN) for the parental lines and the population mean of 240 lines derived from the 'Arina×Forno' cross

Environment	Leaf rust infection (in % infected flag leaf area+RI)[a]					Length of LTN (in mm)				
	Parental lines		'Arina×Forno' population			Parental lines		'Arina×Forno' population		
	'Forno'	'Arina'	SSD Lines	Min	Max	'Forno'	'Arina'	SSD Lines	Min[b]	Max[b]
1999										
Flü99	17MR-MS	61S	32MS-S	0	83S	11.7±2.4[c]	0	8.5±9.9	0	50.0
Grü99	6MR	56S	26MS	0	85S	9.9±8.8	0	10.1±11.6	0	48.5
Haag99	2MR	48S	21MS	0	82S	-	-	-	-	-
2000										
Haag00	3MR	61S	17MS	0	90S	21.4±3.1	0.9±1.5	12.6±9.9	0	47.7
Vouvry00	26S	90S	52S	2MS	100S	47.4±6.8	22.4±3.9	35.3±16.5	4.6	100.2
ZH106	12MS	70S	33S	0.1MR	96S	10.0±1.4	0	6.2±6.0	0	24.5
ZH114	5MS	80S	30S	0.1MR	100S	37.4±5.3	12.0±3.8	25.7±14.8	0.2	63.6
Average 3rd scoring	10MS[d]	65S[d]	30MS	0.1R	88S	23.0±2.4	5.7±0.7	16.4±10.3	0.5	46.7

[a] Leaf rust scorings for the third scoring date were determined using the Cobb scale (%) and the response to infection (RI) on the flag leaf of adult plants

[b] Minima (Min) and maxima (Max)

[c] Standard deviation (±)

[d] Least significant difference, $LSD_{5\%}$, for the average of the third scoring for infected leaf area (%) was 9.5% and for RI 0.6, respectively.

resistance loci on chromosome arms 1BS, 2AL, and 2DS were associated with AUDPC_% and AUDPC_RI across environments (Table 3). Furthermore, one resistance locus affecting the AUDPC_RI and LTN in two environments was detected on chromosome arm 4BS (Table 3; see Electronic Supplementary Material). Finally, the resistance locus on chromosome arm 7BL affected AUDPC_RI (*QLrRI.sfr-7BL.2*) across environments (Table 3) but AUDPC_% (*QLrP.sfr-7BL.2*) in only two environments (see Electronic Supplementary Material). The final simultaneous fit for the explained phenotypic variation (adjusted R^2) for all detected QTLs resulted in 50.3, 53.7, and 45.6% for AUDPC_%, AUDPC_RI, and LTN, respectively (Table 3). The largest and most consistent resistance locus found in all tested environments (see Electronic Supplementary Material) was closely linked to *Xgwm295* on the short arm of chromosome 7D. It explained 32.6, 42.9, and 55.6% of the observed phenotypic variation for AUDPC_%, AUDPC_RI, and LTN, respectively (Fig. 2C, Table 3). The second largest resistance locus consistently detected in five environments (see Electronic Supplementary Material) mapped to the short arm of chromosome 1B close to the centromere and to a QTL for plant height (*QHt.sfr-1BS*) as well as *Xgwm604* (Fig. 2A). It explained 28.0% and 31.5% of the phenotypic variation for AUDPC_% and AUDPC_RI, respectively (Table 3). On chromosome 2D, two resistance loci were found (Table 3). First, the leaf rust resistance locus (AUDPC_% and AUDPC_RI) on the long arm of chromosome 2D overlapped with a QTL for LTN ('Arina' allele) and was detected in five environments for AUDPC_% as well as AUDPC_RI, respectively, and in all tested environments for LTN (see Electronic Supplementary Material). This locus accounted for 11.4, 12.7, and 15.6% of the phenotypic variation for AUDPC_%, AUDPC_RI, and LTN, respectively (Fig. 2B, Table 3). The second region on chromosome arm 2DS revealed one resistance locus (AUDPC_% and AUDPC_RI) proximal to *Xgdm35*, close to *Xgwm296* and *Xgwm261* (Fig. 2B), which was detected in five environments for AUDPC_% and two environments for AUDPC_RI, respectively (see Electronic Supplementary Material). This locus explained 14.8% of the variation for AUDPC_% and 10.3% for AUDPC_RI (Table 3). A

Table 3 Detected quantitative trait loci (QTLs) for leaf rust resistance and leaf tip necrosis (LTN) across all tested environments. For each QTL the corresponding chromosomal location, marker interval, individual R^2 and LOD value is given

Chr.	Marker interval[a]	AUDPC_%[b] (*QLrP.sfr*)		AUDPC_RI[b] (*QLrRI.sfr*)		Leaf tip necrosis (*QLtn.sfr*)		Parental allele
		R^2	LOD[c]	R^2	LOD	R^2	LOD	
1BS	(1) gwm604–OA93	28.0	17.0	31.5	19.7	-	-	'Forno'
2AL	(2) cfa2263c–sfr.BE590525	9.5	5.2	12.0	6.7	-	-	'Forno'
2DS	(3) gdm35–cfd53	14.8	8.2	10.3	5.6	-	-	'Forno'
2DL	(4) glk302–gwm539	11.4	5.9	12.7	6.6	15.6	8.3	'Arina'
4BS	(5) gwm368–gwm540a	-	-	10.7	5.8	-	-	'Forno'
7BS	(6) sfr.BE427461–gwm573b	8.8	4.8	-	-	-	-	'Arina'
7BL	(7) ksuD2–gbxG218b	-	-	15.9	9.0	-	-	'Forno'
7DS	(8) cfd66–gwm1002	32.6	20.6	42.9	29.2	55.6	41.8	'Forno'
Digenic effects, QTL×QTL interaction (%)		1×4 2.2* 1×6 5.5** 2×4 3.3**		1×7 5.4** 1×8 2.7* 2×4 5.7** 2×8 2.2*		-		
Final simultaneous fit for the adjusted R^2 (%) and final LOD		50.3	42.7	53.7	48.3	45.6	32.9	

[a] Marker interval referring to the chromosome in the 'Arina×Forno' map (Paillard et al. 2003)
[b] Resistance QTLs for the area under the disease progress curve (AUDPC) using the Cobb scale (%) and response to infection (RI)
[c] Composite interval mapping (CIM) with a significant LOD threshold of 4.4
*, ** Significant digenic QTL by QTL interactions are indicated by $*P<0.05$ and $**P<0.01$

minor but consistent (see Electronic Supplementary Material) resistance locus mapped to chromosome arm 2AL 2–3 cM proximal to *Xpsr630* accounting for 9.5% and 12.0% of the variation for AUDPC_% and AUDPC_RI, respectively (Table 3).

For both leaf rust scorings, we found several significant digenic QTL by QTL interactions: three for AUDPC_% and four for AUDPC_RI, respectively (Table 3). The resistance locus on chromosome arm 1BS was involved in four out of these seven interactions, whereas the locus on 2AL interacted three times (Table 3). In five of these interactions, the resistance loci on 1BS and 2AL simultaneously interacted with resistance loci located on chromosome arms 7DS and 2DL which are both involved in conferring resistance for three traits (AUDPC_%, AUDPC_RI, LTN; Table 3).

Discussion

Leaf rust resistance loci segregating within the 'Arina×Forno' population

The most consistent and largest leaf rust resistance locus (*QLrP.sfr-7DS* and *QLrRI.sfr-7DS*) was detected on the short arm of chromosome 7D overlapping with a major locus for LTN (*QLtn.sfr-7DS*). This resistance locus maps at a very similar location to *Ltn*/*Lr34* in other wheat populations (Nelson et al. 1997; Suenaga et al. 2003). We have observed the specific *Lr34* phenotype expressed on the flag leaf in some lines of the population , i.e., the presence of LTN and a pustule gradient (Kolmer 1996). There was also a strong phenotypic correlation between leaf rust resistance and the presence of LTN (Singh 1992a; Messmer et al. 2000). Thus, our findings strongly suggest that the major resistance locus on 7DS corresponds to the *Ltn*/*Lr34* gene or a winter wheat allele of it. Pedigree data from *cv.* 'Forno' revealed that one parent is the German *cv.* 'Kormoran' which also exhibits LTN. 'Kormoran' is descended from the French variety 'Cappelle Desprez' which was broadly used in Europe because it conferred a durable and adult plant yellow rust resistance (McIntosh 1992), but also a partial leaf rust resistance (Poyntz and Hyde 1987; McIntosh 1992; Denissen 1993; McIntosh et al. 1995b). There has been evidence for the presence of *Lr34* in the French winter wheat germplasm (Dyck 1994) as well as in 'Cappelle Desprez' (McIntosh 1992; McIntosh et al. 1995b).

In an earlier study (Messmer et al. 2000) analyzing the quantitative and durable leaf rust resistance in the 'Forno×spelt' population, no QTL in the *Lr34* chromosomal region was described. This is possibly due to lack of polymorphism on 7DS in this population.

Beside the major leaf rust resistance locus on 7DS, another major leaf rust resistance locus was identified on chromosome arm 1BS. *QLr.sfr-1BS* was not associated with LTN, however, it is strongly involved in QTL by QTL interactions and was already discovered with a smaller effect in the 'Forno×spelt' population (Messmer et al. 2000). As *QLr.sfr-1BS* proved to be effective in different genetic backgrounds and was mapped very close (less than 1 cM) to *Xgwm604*, this marker would be suitable for marker assisted selection for leaf rust resistance. Other mapping studies have identified the leaf rust resistance gene *Lr26*, which was derived from a 1B/1R wheat-rye translocation, on chromosome arm 1BS (McIntosh et al. 1995a; Mago et al. 2002). Furthermore, a major leaf rust resistance QTL located on 1BS was also detected in the CIMMYT spring wheat germplasm (William et al. 1997). Later studies revealed that this locus was different from the nonhypersensitive adult plant leaf rust resistance gene *Lr46* which is located on 1BL

close to *Xwmc44* (Suenaga et al. 2003; William et al. 2003).

The leaf rust resistance locus on the short arm of chromosome 2D mapped approximately 3 cM proximal to *Xgdm35,* peaking at *Xgwm296* in a region where many *Lr* genes are located. The *Aegilops tauschii*-derived leaf rust resistance gene *Lr39* (=allelic to *Lr41*) (Singh et al. 2003) was recently mapped distal to *Xgdm35* whereas *Lr2* and the APR gene *Lr22* were located proximal to *Xgdm35* and *Xgwm210* (Raupp et al. 2001).

On the distal end of the long arm of chromosome 7B, we found a minor leaf rust resistance locus in a cluster of eight markers within 5 cM (Paillard et al. 2003). One of the markers within the cluster codes for a thaumatin-like protein (*Xpwir232*), which was detected as the major leaf rust resistance locus in the 'Forno×spelt' population (Messmer et al. 2000). In the wheat cross 'Arina×Forno' we have found only a small effect attributable to this locus. During a wheat leaf rust survey for western Europe it was found that 'Forno' might possess the leaf rust resistance gene *Lr14a* (Park et al. 2001), which was also mapped in the wheat consensus map to the distal end of 7BL (Gale et al. 1995).

Two other minor leaf rust resistance loci on chromosome arms 2DL and 4BS were mapped in association with LTN. The resistance allele from 2DL derived from the susceptible parent 'Arina' affected mostly the pustule size (RI). Compared to other highly leaf rust susceptible varieties such as 'Morocco', *cv.* 'Arina' displays a reduced pustule size which might be due to a resistance correlating with small 'leaf tip necrosis' (on average 6 mm). Compared to LTN in 'Forno' (23 mm), this 'leaf tip necrosis' is clearly distinguishable from the LTN phenotype.

Another minor leaf rust resistance locus on chromosome 2AL close to *Xpsr630,* which was not mapped in association with LTN, consistently contributed to resistance. This locus interacted exclusively with resistance loci on 7DS and 2DL which were also involved in the expression of LTN. So far, there are no indications for the presence of any *Lr* genes on 2AL, but resistance genes against other diseases such as *Yr1*, *Pm4*, and *Sr21* were mapped to the same chromosome arm (The et al. 1979; McIntosh and Arts 1996).

Leaf rust resistance and LTN

The fact that we found a high phenotypic correlation between leaf rust resistance and the presence of LTN in our population as well as the lack of highly susceptible lines exhibiting LTN supports the hypothesis of the pleiotropic action of a single gene. Furthermore, all genetically mapped *Ltn* loci overlapped with leaf rust resistance loci and we detected several significant epistatic QTL by QTL interactions which involved *Ltn* and leaf rust resistance loci, suggesting that these genes might interact in the same resistance pathway. Based on previous observations (Messmer et al. 2000) and our findings for the association between LTN and leaf rust resistance in the 'Arina×Forno' population, we postulate that *Ltn* is pleiotropic to *Lr34* rather than tightly linked with *Lr34*. For future investigations it remains to be shown whether the effect causing LTN is responsible for the partial leaf rust resistance.

Postulated leaf rust resistance in *cv.* 'Forno'

We propose that the quantitative and durable leaf rust resistance in the Swiss *cv.* 'Forno' is based on a combination of three different types of genetic resistance: The first and main resistance effect is due to the major resistance locus on chromosome arm 7DS which may be *Lr34* associated with *Ltn*. The second type of resistance is represented by other partial leaf rust resistance loci such as *QLr.sfr-1BS* and *QLr.sfr-2AL,* which are not associated with LTN. Finally, the action of various major disease resistance genes like *Lr2* or *Lr22* on 2DS and *Lr14a* (7BL) constitutes the third resistance mechanism. The combination and interaction of all these genes provides a high level of durable leaf rust resistance in *cv.* 'Forno'.

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