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ORIGINAL PAPER

Durable resistance to stripe rust is due to three specific resistance genes in French bread wheat cultivar Apache

S. Paillard · G. Trotoux-Verplancke · M.-R. Perretant · F. Mohamadi · M. Leconte · S. Coëdel · C. de Vallavieille-Pope · F. Dedryver

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Abstract Quantitative resistance is postulated to be more durable than qualitative (R-gene mediated) resistance, which is usually quickly overcome by the pathogen population. Despite its wide use for nearly 10 years in France, the French bread wheat cultivar Apache remains resistant to stripe rust. Here, we investigated the genetic architecture of cv. Apache resistance to examine whether its durability could be explained by quantitative characteristics. We identified quantitative trait loci (QTL) by composite interval mapping of disease progress data recorded throughout 4 years of field assays. These assays included inoculation with three different pathotypes on a segregating population originating from a cross between cv. Apache and cv. Taldor, a French cultivar susceptible to stripe rust. Three QTLs derived from Apache, *QYr.inra-2AS, QYr.inra-2BL* and *QYr.inra-4B*, were detected. Each of these QTLs contributed between approximately 15 and 69 % of the phenotypic variance and corresponds to a race-specific resistance gene. We showed that *QYr.inra-2AS* and *QYr.inra-2BS* map to the positions of *Yr17* and *Yr7*, respectively, whereas *QYr.inra-4B* corresponds to an adult plant resistance gene. Our results demonstrate that a combination of two or more race-specific resistance genes can confer durable resistance provided that it is properly managed at a continental level. Race-specific resistance genes should not be removed from breeding programs provided that they are properly managed.

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S. Paillard (✉) · G. Trotoux-Verplancke · F. Mohamadi · F. Dedryver
INRA UMR 118, Amélioration des Plantes et Biotechnologies Végétales, Domaine de la Motte, BP 35327, 35653 Le Rheu cedex, France
e-mail: paillard@rennes.inra.fr

M.-R. Perretant
INRA UMR 1095, 63100 Clermont-Ferrand, France

M. Leconte · C. de Vallavieille-Pope
INRA BIOGER-CPP, UR 1290, BP 01, 78850 Thiverval-Grignon, France

S. Coëdel
INRA UMR 118 Biogenouest, Plateforme Genotypage, Domaine de la Motte, BP 35327, 35653 Le Rheu cedex, France

Introduction

Stripe rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., is a devastating disease of wheat (*Triticum aestivum* L.) in cool and humid production regions (Zadoks 1961). In most wheat-producing areas, yield losses caused by stripe rust are 10–70 % depending on the susceptibility of the cultivar, earliness of the initial infection, rate of disease development and duration of disease (Chen 2005). The use of resistant cultivars is the most effective, economical and environmentally friendly means to control stripe rust. Resistance to stripe rust can be categorized as all-stage resistance (also known as seedling resistance [SR]), which can be detected at the seedling stage but is expressed at all stages of plant growth, or as adult plant resistance (APR), expressed at later stages of plant development (Chen 2005). Seventy validated and tentative resistance genes, designated *Yr*, were reported by Chen (2005) and McIntosh et al. (2008). Seedling resistance is generally qualitatively inherited, race-specific, and, thus, easily overcome by new virulent races of the rust fungus (Johnson 1981). Although this may also be the case for APR genes (Johnson 1992), APR is generally quantitatively inherited, non-race-specific and durable (Börner et al. 2000; Johnson 1984).

The present work describes the genetic analysis of resistance to stripe rust in the French bread wheat cv. Apache. Apache still shows effective resistance in France despite being the most or one of the most widely grown wheat cultivars (from 10 to 24 % of total wheat acreage in France) for nearly 10 years (2002 to date) in an environment favourable for stripe rust epidemics. Two major epidemics occurred in northern France in the periods 1999–2002 and 2007–2009, due to successive acquisitions of virulences to the resistance genes *Yr17* and *Yr32*, either by acquisition of the virulence in the previous dominant pathotype or by incursion/selection of news pathotypes (de Vallavieille-Pope et al. 2012). Each epidemic was characterized by the breakdown of a newly deployed or re-deployed resistance gene. New pathotypes were detected in the years following the absence of epidemics (no epidemic in the periods 1996–1997 and 2005–2006), as in the case of the virulence *V17* in 1998 and the virulence *V32* in 2007 (de Vallavieille-Pope et al. 2012). Apache was suspected to possess the *Yr17* gene because it tested positive for SC-Y15 SCAR (Robert et al. 1999), a marker that is specific to the cluster of rust resistance genes *Yr17*, *Lr37* and *Sr38*, which is located on an introgression from *Aegilops ventricosa* into the 2AS chromosome of wheat (Ambrozkova et al. 2002). Furthermore, Blaszczyk et al. (2004) demonstrated the presence of *Lr37* in Apache using seedling resistance tests. But as Apache was not susceptible to stripe rust isolates carrying virulence for *Yr17*, and exhibited durable resistance, this resistance was suspected to be quantitatively inherited. We thus investigated which quantitative trait loci (QTL)/gene associations allow the resistance of Apache to remain effective. In this study, we show that Apache carries a gene for APR located on the short arm of chromosome 4B. This gene confers resistance only at the adult plant stage and is race-specific, as it is defeated by two of the tested *P. striiformis* f. sp. *tritici* isolates. We also show that Apache carries the *Yr7* SR gene and confirm the presence of *Yr17* in this cultivar. Both *Yr7* and *Yr17* were defeated in France, in 1983 and 1998, respectively. The efficiency of Apache resistance despite its extensive use in France for nearly 10 years is discussed.

Materials and methods

Plant material

Two French elite lines of bread wheat *T. aestivum* cv. Apache and cv. Taldor were crossed in 1999 at Institut National de la Recherche Agronomique (INRA) in Clermont-Ferrand in order to produce a population of doubled haploid (DH) lines. This population was obtained by intergeneric crossing of F1 plants with maize, using a method derived from that proposed by David et al. (1999) for durum wheat and modified slightly for use in bread wheat. Specifically, after excision from the immature grains, haploid embryos were first cultivated in Petri dishes on a MS-like medium until plantlet regeneration. Then, plantlets were transferred into pots containing agarose solidified MS medium + vitamins (ref M 0222- Kalys, impasse du Teura Fr 38190 BERNIN) with the rhizogen hormone ANA added at a concentration of 0.2 mg l^{-1}. The crown (tiller basis) of plantlets was then incubated in a colchicine solution (ref C 1305 25 also provided by Kalys) in order to induce chromosome number doubling in meristem cells. Incubation lasted 4 h at 26 °C under artificial light in a 2 % solution. Six hundred and twenty recombinant plants, all different and fertile, were obtained, suitable for different survey purposes. Pedigrees and characteristics of both parental lines are available at http://genbank.vurv.cz/wheat/pedigree/.

Pathogen materials

The *P. striiformis* f. sp. *tritici* pathotypes used in this study are part of the stripe rust collection held by C. de Vallavieille-Pope at UMR Bioger, INRA Grignon. Two pathotypes with wide virulence spectra were chosen to detect the *Yr17* gene: the 237E141 pathotype, which possesses virulence to *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr9, Yr11, Yr25*, *YrSU* and avirulence for *Yr5, Yr7, Yr8, Yr10, Yr12, Yr13*, *Yr14, Yr17* and *Yr32* and the 237E141V17 pathotype, which contains an additional virulence factor to *Yr17* (Bayles et al. 2000). The third pathotype 106E139 was chosen to overcome the *Yr7* gene and possesses virulence to *Yr2*, *Yr3*, *Yr4*, *Yr7, Yr11, Yr25* and *YrSU* and avirulence for *Yr1*, *Yr5*, *Yr6*, *Yr8, Yr9, Yr10, Yr12*, *Yr13*, *Yr14, Yr17* and *Yr32*.

Spores of each pathotype were increased on susceptible genotypes: cv. Récital for both pathotypes 237E141 and 237E141V17 and cv. Talent for the 106E139 pathotype. The plants of each genotype were grown in a growth chamber at 20 °C and with a 16 h light/8 h dark photoperiod until the second leaf appeared. The plants were then inoculated by spraying the leaves with a spore suspension (in mineral oil: Soltrol® 170 Isoparaffin, Chevron Phillips Chemical Company LP, The Woodlands, TX, USA) of one pathotype. The inoculated plants were first placed in a growth chamber at 12 °C and 100 % relative humidity, in the dark, for 24 h to ensure spore germination and penetration and then returned to a growth chamber and grown at 8 °C for 3–5 weeks with a 8 h light/16 h dark photoperiod. Spores were collected 3–5 weeks after inoculation.

Spores collected on susceptible genotypes during field experiments were tested after each field assessment, on a set of 21 stripe rust standard differentials at the second-leaf stage (de Vallavieille-Pope et al. 1990, 2000) to confirm

the virulence spectrum of *P. striiformis* f. sp. *tritici* pathotype. Twenty-four hours after the inoculation of the 21 differential genotypes, the plants were placed in a growth chamber and grown at 20 °C for 15 days with a 16 h light/8 h dark photoperiod. At 12 and 15 days after inoculation, symptoms of stripe rust resistance were recorded using the scale described by McNeal et al. (1971).

Field trials and field assessment of resistance

One hundred and eighty-two DH lines, their parents and differential hosts (*Yr1* to *Yr14, Yr16, Yr17, Yr32, Su* and *SD*) were evaluated in the field at the INRA experimental farm in Le Rheu (near Rennes-35, France) in 2002 with the *P. striiformis* f. sp. *tritici* 237E141 pathotype; in 2006 and 2008 with the 237E141V17 pathotype; and in 2009 with the 106E139 pathotype. In every experiment, 12 seeds from each line were sown in a row at the end of October and arranged in a completely randomized block design, with two replicates. Susceptible disease spreader plants were planted in every third row: the cv. Récital (*Yr6*) in 2002, 2006 and 2008 and a mixture of cultivars (cvs: Lee, Talent, Thatcher carrying *Yr7*) in 2009. Additionally, seedlings at the two-leaf stage were artificially inoculated with *P. striiformis* f. sp. *tritici* in a growth chamber and then transplanted to the experimental plot when leaves were totally covered by stripe rust pustules. To ensure a high level of disease pressure, two or three inoculated plants were planted in 30 % of spreader rows during November, January, February and early March of each year.

Stripe rust scores were recorded on three or four separate occasions in 2002/2006 and 2008/2009, respectively: at the time of rust appearance (N1) corresponding to the growth stage, DC32 (Zadoks et al. 1974), and after each cycle of pathogen multiplication on the susceptible parent Taldor (N2, N3 and N4), corresponding, respectively, to the growth stages, DC39, DC64 and DC79, of Taldor. The scoring method considered the percentage of leaf area covered by pustules on the three most heavily infested leaves of each plant, using a 0–12 scale (Table 1). The estimation of the area under the disease progress curve (AUDPC) was described by Mallard et al. (2005). At the end of each experiment, rusted leaves were randomly collected in the experimental field. Differential hosts were inoculated with the collected spores to confirm the identity of the pathotype, using seedling-stage assessment.

Statistical analysis

Statistical analysis of AUDPC traits was performed using the SAS statistics package (SAS Institute, Raleigh, NC). Genotype, replication and year effects obtained with each pathotype were tested using analysis of variance (ANOVA) by the PROC GLM procedure. For each year, homogeneity of phenotypic variances between replications and genotypes was verified using Bartlett's test, and normality of residual distributions was verified using the PROC UNIVARIATE procedure and Shapiro's w statistic. Within each year, broad-sense heritability for stripe rust resistance was estimated from the ANOVA using the formula: $h^2 = \sigma^2_G / \left[\sigma^2_G + \left(\sigma^2_e / r\right)\right]$, where σ^2_G is the genetic variance, σ^2_e is the residual variance and r is the number of replications. For all tests, a probability level $P < 0.05$ was used to declare significance.

The segregation ratio between the resistant and susceptible response groups was tested for its compatibility with mono or digenic expectations using χ^2 statistic.

Map construction

The parental lines Apache and Taldor were screened with over 417 microsatellites (SSR) markers including BARC (http://www.scabusa.org), CFD and CFA (Guyomarc'h et al. 2002; Sourdille et al. 2001, 2003), CNL (Yu et al. 2004), GWM (Röder et al. 1998), GPW (Génoplante) and WMC (Somers et al. 2004) markers. Two hundred and forty-one polymorphic SSR markers were then mapped against 183 DH lines from the Apache × Taldor cross. PCR reactions were performed classically using the *Taq* polymerase provided by Qiagen and M13-elongated primers. Labelling products were mainly provided by Applied Biosystems. PCR products were then electrophoresed on an ABI 3100 16-capillary DNA sequencer (Applied Biosystems, Foster City, CA) using the Pop7 polymer. The results were finally analysed with the software package GenMapper version 3.7 provided by the Applied Biosystem. Additionally, a SCAR marker distinctive of *Yr17* gene, SC-Y15 described by Robert et al. (1999) was mapped in this study.

For each segregating marker, a Chi-square analysis ($\alpha = 0.01$) was performed to test for deviation from the expected segregation (ratio 1:1). Linkage analysis was performed with the package MAPMAKER/EXP ver. 3.1 (Lander et al. 1987; Lincoln et al. 1992). Linkage groups

Table 1 Scale used for stripe rust ratings at the adult plant stage

Percentage of leaf area affected by disease	0	0.3	0.7	2	8	12	16	24	33	50	66	82	100
Scale	0	1	2	3	4	5	6	7	8	9	10	11	12

were determined using a logarithm of odds (LOD) score of 7.0 and a maximum distance between two markers of 40 cM. For each linkage group, the best marker loci order was determined using three-point and multi-point analyses, enlisting the ORDER or the TRY commands. Genetic distances were calculated using the Haldane mapping function (Haldane 1919). Linkage groups were assigned to chromosomes in comparison with the International Triticeae Mapping Initiative (ITMI) map (Röder et al. 1998) and wheat consensus map (http://wheat.pw.usda.gov).

QTL analysis

A one-way ANOVA with a probability level of $P < 0.001$ was employed to identify markers with significant effects on resistance. QTL detection was carried out by composite interval mapping (CIM) (Zeng 1993, 1994) using QTL CARTOGRAPHER software ver. 2.5 (Wang et al. 2010). A forward–backward stepwise regression was performed to choose cofactors before QTL detection by CIM. Ten cofactors with the highest F value were taken into account. A window size of 10 cM around the test interval was chosen for all analyses. Permutation tests were carried out to identify the appropriate significant LOD thresholds for each trait to identify a QTL. After 1,000 permutations, the critical LOD threshold was LOD = 2.8. The QTL × QTL interactions and percentage of phenotypic variation explained by the whole model (total R^2) were determined using multiple interval mapping (MIM). For MIM analysis, the QTL peaks above the critical LOD thresholds from the CIM analysis were used as the initial model. New QTLs were added to the current model. The proportion of phenotypic variation explained by individual QTL and by the whole model (total R^2), the additive and dominant effects, and the QTL–QTL interaction were estimated using the SUMMARY option of MIM.

Results

Phenotypic assessments of stripe rust infection

The analysis of spores collected at the end of each field experiment revealed that no contamination with other pathotypes occurred during the growing season. The frequency distribution for AUDPC in all four trials was continuous (Fig. 1). All Apache plants were completely resistant to all three pathotypes (AUDPC = 0). The susceptible parent Taldor showed maximum AUDPCs of 215, 238, 286 and 370 in 2002, 2006, 2008 and 2009, respectively. For each pathotype, within-year and over-year heritability values for the AUDPC were high (Table 2). ANOVA (Table 2) showed significant genotype effects for AUDPC within each year (2002, 2006, 2008 and 2009) and over years with the same pathotype (2006 and 2008). No significant replicate or year effect was detected with the same pathotype. High levels of correlation were observed between replicates of each year (0.95 in 2002, 0.93 in 2006, 0.95 in 2008 and 0.93 in 2009) and between years for the same pathotype (0.89 for 2006/2008). Consequently, the means of the two replicates were used each year for QTL detection.

Map construction and QTL identification

A total of 310 markers were found to be polymorphic between Apache and Taldor. Of these, 241 were successfully used to genotype and differentiate individual DH lines. A genetic map with a genetic length of 1,249 cM was constructed that represented all 21 chromosomes as 36 linkage groups. Significant segregation distortion was observed for 24 markers. No distorted markers were observed on the chromosomes carrying the resistance QTLs.

QTLs for stripe rust resistance were detected separately in each of the 4 years using CIM analysis on the AUDPC data. For the 2002 assessments, based on the 237E141 pathotype's avirulence to *Yr7* and *Yr17*, two QTLs with additive effects were detected (Table 3; Fig. 2). These two QTLs were localized on chromosomes 2AS and 2BL, respectively, and explained together more than 45 % of the phenotypic variance. The resistance of the QTL *QYr.inra-2AS*, explaining 25.9 % of the phenotypic variation, was conferred by *A. ventricosa* alleles, found within the *Yr17-Lr37-Sr38* translocation into the *Xbarc212-XSC-Y15* interval of the resistant parent Apache. Thus, *QYr.inra-2AS* corresponds to the *Yr17* gene. *QYr.inra-2BL* explained 24.3 % of the phenotypic variation with the positive allele for resistance being contributed by Apache.

The 237E141V17 pathotype, which is virulent to *Yr17*, was used in the 2006/2008 assessments: only the major QTL *QYr.inra-2BL* was detected and explained over 60 % of the phenotypic variation (Table 3; Fig. 2). The *Yr17* gene associated with the introgression of *A. ventricosa* into the Apache chromosome 2AS was no longer detected. One QTL, *QYr.inra-2AL*, with a resistance allele contributed by Taldor was also identified in 2006 and 2008. This QTL had a minor effect (R^2 = 2–3.6 %).

For the 2009 assessments, based on the pathotype 106E139, which is avirulent to *Yr17*, two gene/QTLs with additive effects were detected. The *Yr17* gene conferred by *A. ventricosa* alleles and previously detected in 2002 explained a large proportion of the phenotypic variation (R^2 = 22.6 %). Compared to the 2002, 2006 and 2008 data, a new QTL was detected on the chromosome 4B: *QYr.inra-4B*, with resistance alleles conferred by Apache,

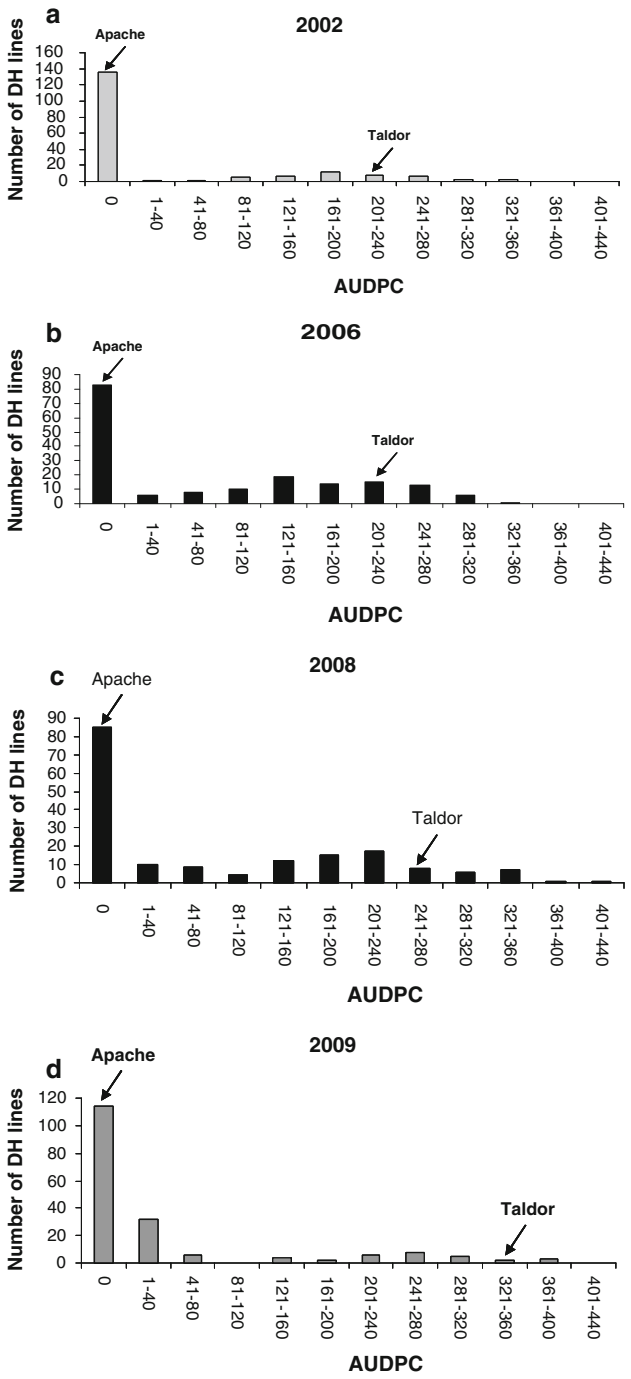

Fig. 1 Phenotypic distribution of stripe rust intensity area under the disease progress curves (AUDPC) for the DH lines derived from a cross between Apache × Taldor. The DH lines were infected in 2002, 2006/2008 and 2009, respectively, with the *Puccinia striiformis* f. sp. *tritici* pathotypes 237E141, 237E141V17 and 106E139

explaining 14.5 % of the phenotypic variation. The QTL *QYr.inra-2BL*, which was detected with the two other pathotypes, was no longer detected with pathotype 106E139 described as virulent against *Yr7*. This QTL maps to the previously mapped position of the two allelic genes *Yr5* and *Yr7* (Zhang et al. 2009) and corresponds to the region (10.5–13.3 cM) of the linked marker locus *Xgwm501* (Sun et al. 2002; Cheng and Chen 2010). Furthermore, cv. Apache seedlings inoculated in a fully confined S3-type growth chamber were susceptible to an isolate carrying *V7* and *V17* which is not present in France (Table 4). We can thus postulate that *QYr.inra-2BL* corresponds to *Yr7* SR gene.

ANOVA results confirmed that all markers included within the QTL intervals were significantly ($P < 0.001$) associated with stripe rust resistance (data not shown).

The epistatic effects of QTLs were also evaluated using MIM analysis and showed that some interactions between different QTLs or markers were significant in 2002, 2008 and 2009. In 2002, significant interactions were detected between *Xcfd36b*, a marker of *A. ventricosa* introgression into wheat chromosome 2AS, and *QYr.inra-2BL-Yr7* QTL. This interaction explained 24.6 % of the variance. In 2008, one interaction (4 % of the variance) was observed between *QYr.inra-2BL-Yr7* and one locus, *Xgpw4496* (2AL), which had been previously linked to the *QYr.inra-2AL* QTL. In 2009, an interaction explaining 24 % of the variance was detected between the *QYr.inra-2AS* QTL linked to the *A. ventricosa* introgression into 2AS and the locus *Xgwm6*, a marker of *QYr.inra-4B* QTL. The combined additive and epistatic effects accounted for 78, 83, 88 and 65 % of the total phenotypic variance in 2002, 2006, 2008 and 2009, respectively.

Segregation analysis for reaction to stripe rust under field conditions

Chi-squared analysis (Table 5) showed that resistant and susceptible DH lines segregated at a ratio of 1:1 or 3:1, supporting the hypothesis of a single gene or two genes, respectively. The data showed a goodness-of-fit for segregation of one gene and two genes in 2006/2008 (237E141V17 pathotype) and in 2002/2009 (237E141 and 106E139 pathotypes), respectively. Chi-squared analyses (Table 5) showed that two major genes segregated independently in 2002 (237E141 pathotype) and 2009 (106E139 pathotype).

The DH lines derived from the Apache × Taldor cross were classified, based on their reaction against the three stripe rust pathotypes (Table 6). Five phenotypic classes were described. Forty-seven and 24 DH lines were susceptible to only one pathotype, 237E141V17 (2006/2008) and 106E139 (2009), respectively. These lines were completely resistant (AUDPC = 0) to the two other pathotypes. Twenty-two DH lines were susceptible to the 237E141 and 237E141V17 pathotypes in 2002 and 2006/2008, respectively. These 22 lines were only resistant in 2009 against the 106E139 pathotype, but all did not

Table 2 Analysis of variance of genotype, replicate and year effects on the area under the disease progress curves and the proportion of phenotypic variation for stripe rust intensity among DH lines generated from a cross between Apache and Taldor cultivars

Year	MS	*F* value	*P* value	h^2
2002				
Genotypes	16,060	79.59	<0.0001	0.987
Replicates	1.5	0.01	0.9315	
Error	201			
2006/2008				
Genotypes	47,132	65.69	<0.0001	0.984
Replicates	255	0.36	0.5508	
Years	1,680	2.34	0.1265	
Error	717			
2009				
Genotypes	19,043	57.18	<0.0001	0.982
Replicates	980	2.94	0.0881	
Error	333			

The DH lines were infected in 2002, 2006/2008 and 2009, respectively, with the *Puccinia striiformis* f. sp. *tritici* pathotypes. 237E141 (*v1, 2, 3, 4, 6, 9, 25, SU*), 237E141V17 (*v1, 2, 3, 4, 6, 9, 17, 25, SU)* and 106E139 (*v2, 3, 4, 7, 25, SU*)

MS mean square, h^2 broad-sense heritability

show complete resistance under field conditions ($0 \leq$ AUDPC ≤ 22) which corresponded to a slight infection only for the N1 scoring (2 % of leaf area affected by disease). Thus, these 22 lines were also tested with the 106E139 pathotype at the second-leaf stage, using the same protocol as that described for the pathotype characterization of spores collected during field assessments. These lines, as well as the susceptible parent Taldor, exhibited an infection type of 8–9, whereas the resistant parent Apache showed an infection type equal to 2. This demonstrates that Apache carries a specific APR. This APR gene was only effective in 2009 against the 106E139 pathotype (phenotypic class D, Table 6) like the QTL *QYr.inra-4B* with major effect. Thus, the QTL *QYr.inra-4B* probably corresponds to the APR gene.

The interpretation of the responses of each phenotypic class × pathotype interaction under field conditions allowed assigning some postulated genotypes to each phenotypic class (Table 6). The *Yr7*, *Yr17* and APR gene presence in each phenotypic class was also confirmed by the use of markers closely linked to the positive alleles for resistance contributed by Apache. The *Yr17* gene, which was effective in 2002 against the 237E141 pathotype, was overcome in 2006/2008 with the 237E141V17 pathotype (phenotypic class C, Table 6). This breakdown was much probably due to the extra virulence *V17*. This gene was also effective in 2009 against another pathotype (106E139), which does not possess this *V17* virulence (phenotypic class C, Table 6). The *Yr7* gene only conferred resistance to pathotypes 237E141 (2002) and 237E141V17 (2006/2008) which do not carry virulence *V7* (phenotypic class B, Table 6). These two major genes segregated independently in the DH population when confronted with the pathotype 237E141 in 2002 (χ^2, Table 5). The APR gene/*QYr.inra-4B* was only effective against the 106 E139 pathotype in 2009 (phenotypic class D, Table 6) and segregated with the postulated *Yr17* gene (χ^2, Table 5 and phenotypic class C, Table 6). The 66 DH lines in phenotypic class A (Table 6) were resistant to all three pathotypes, and their genotypes were either *Yr7–Yr17* or *Yr7*-APR gene or *Yr7–Yr17*-APR gene. The 22 DH lines in phenotypic class E (Table 6) were susceptible to all three pathotypes and did not carry positive allele for the resistance contributed by Apache.

Table 3 Summary of resistance QTLs against yellow rust

Year	Chromosome[a]	Origin[b]	Peak position (cM)	Nearest marker locus	LOD	R^2
2002	2AS (*Yr17*)	Ap	10.67	*Xcfd36b*	18.39	25.95
	2BL (*Yr7*)	Ap	62.94	*Xcfd267*	17.34	24.26
2006	2AL	Ta	78.55	*Xgpw4496*	3.05	2.02
	2BL (*Yr7*)	Ap	58.71	*Xcfd73a*	52.38	69.71
2008	2AL	Ta	85.53	*Xgpw4496*	4.27	3.87
	2BL (*Yr7*)	Ap	64.54	*Xcfd267*	45.27	62.77
2009	2AS (*Yr17*)	Ap	12.32	*Xcnl127*	13.62	22.57
	4B	Ap	12.34	*Xgwm6*	9.17	14.54

QTLs were identified by composite interval mapping on area under the disease progress curves (AUDPC) for an Apache × Taldor cross. Three different *Puccinia striiformis* f. sp. *tritici* pathotypes, 237E141, 237E141V17 and 106E139, were used in 2002, 2006/2008 and 2009, respectively

[a] Chromosomal location of each QTL

[b] Origin of resistance allele (*Ap* Apache, *Ta* Taldor)

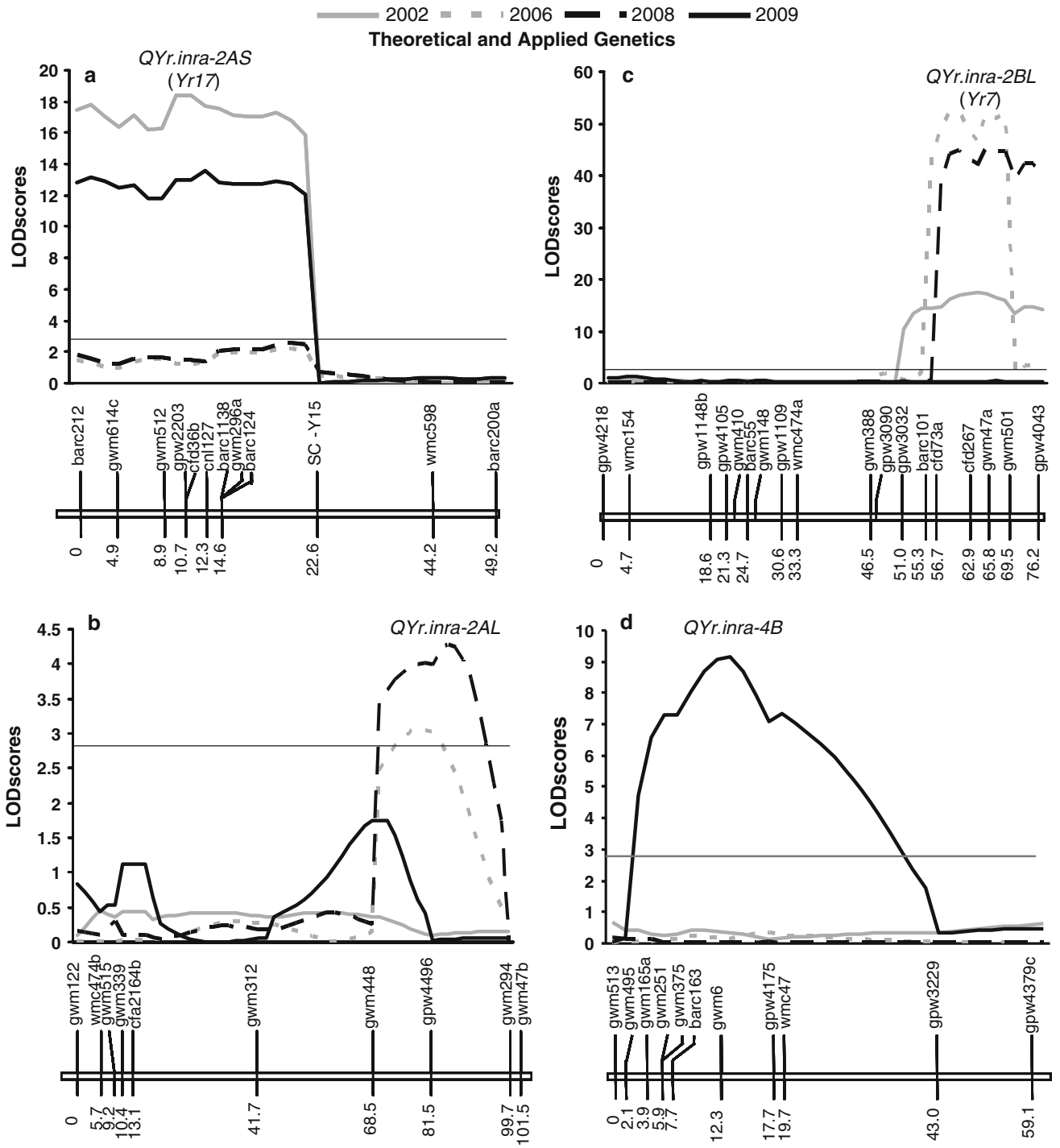

Fig. 2 Quantitative trait loci identified in 2002, 2006, 2008 and 2009 with the *Puccinia striiformis* f. sp. *tritici* pathotypes 237E141(2002), 237E141V17 (2006, 2008) and 106E139 (2009) and mapped on linkage groups corresponding to chromosomes **a** 2AS, **b** 2AL, **c** 2BL, and **d** 4B. QTLs were detected using the area under the disease progress curve data. The *horizontal line* indicates significant LOD thresholds (2.8). The positions of markers are shown along the chromosomes and the distance between marker loci is in centimorgan

Discussion

Statistical analyses of the stripe rust resistance data observed under field conditions showed high levels of repeatability within each year and between years for a same pathotype (heritability, ANOVA and correlation). This reflects the accuracy and reproducibility of the experimental conditions and scoring method used for stripe rust resistance evaluation under field conditions. For the 237E141V17 pathotype, which was inoculated both in

Table 4 Results for Apache tests with different isolates at the seedling stage (de Vallavieille-Pope et al. 1990, 2000)

Pathotypes (virulence)	Interaction
6E16AV9 (*v2, 6, 7, 8*)	−
6E16V9 (*v2, 6, 7, 8, 9, 25, 27, A*)	−
40E8 (*v3, 25*)	−
43E138 (*v1, 2, 3, 7, 25*)	−
106E139 (*v2, 3, 4, 7, 25, SU*)	−
109E141 (*v1, 2, 3, 4, 6, 25, SU*)	−
169E136V17 (*v1, 2, 3, 9, 17, 25, A*)	−
232E137 (*v2, 3, 4, 9, 25, SU*)	−
233E169V17 (*v1, 2, 3, 4, 9, 17, 32, 25, SU*)	−
237E141 (*v1, 2, 3, 4, 6, 9, 25, SU*)	−
237E141V17 (*v1, 2, 3, 4, 6, 9, 17, 25, SU*)	−
239E143V17 (*v1, 2, 3, 4, 6, 7, 9, 17, 25, SU*)	+

+ Compatible reaction (susceptibility), − incompatible reaction (resistance)

2006 and 2008, the frequency distribution of stripe rust scores showed more classes of AUDPC in 2008 than in 2006. This result was probably due to the higher number of scores and days between the first and last scores in 2008 (4 and 40, respectively) than in 2006 (3 and 30, respectively). Nevertheless, the AUDPC means were similar in 2008 (mean = 94) and 2006 (90).

The present study showed that Apache's resistance is under polygenic control. The CIM analysis identified resistance QTLs, including major and race-specific genes *Yr7* and *Yr17*. The QTL *QYr.inra-2AS*, which was only detected with the pathotypes 237E141 and 106E139 (avirulent against *Yr17)*, was associated with marker alleles corresponding to the introgression of *A. ventricosa* carrying *Yr17* into Apache chromosome 2AS. The QTL *QYr.inra-2BL*, detected with the pathotypes 237E141 and 237E14 1V17, was overcome in 2009 by the 106E139 pathotype, described as virulent against *Yr7*, and mapped to the previously mapped position of *Yr7* gene (Sun et al. 2002; Cheng and Chen 2010).

A seedling test performed with the 106E139 pathotype on the 22 lines, which showed resistance in the field with only this pathotype, indicated that Apache also carries a race-specific APR. The *QYr.inra-4B* QTL was only efficient against the 106E139 pathotype, and thus corresponds to the APR gene. Previous studies reported three QTLs, explaining 3.0–12 % of the stripe rust resistance phenotype, on chromosome 4B. These QTLs were some 5 cM away from the peak of *QYr.inra-4B* (*Xgwm6*) and have been found in the cvs Oligoculm (Suenaga et al. 2003), MV17 (Badakhshan et al. 2008) and Guardian (Melichar et al. 2008). In this chromosomal region, a major stripe rust resistance QTL ($R^2 = 28.90$ %) was also identified in

Table 5 Segregation of the reaction to stripe rust in DH progeny of an Apache × Taldor cross in field conditions

Pathotypes (virulence) year	Observed number of DH lines			Expected ratio	χ^2	*P*
	Resistant	Susceptible	Total			
237E141 (*v1, 2, 3, 4, 6, 9, 11, 25, SU*) 2002[a]	137	44	181	3:1	0.046	0.83
237E141V17 (*v1, 2, 3, 4, 6, 9, 11, 17, 25, SU*) 2006[a]	83	92	175	1:1	0.463	0.49
237E141V17 (*v1, 2, 3, 4, 6, 9, 11, 17, 25, SU*) 2008[a]	85	90	175	1:1	0.143	0.70
106E139 (*v2, 3, 4, 7, 11, 25, SU*) 2009[b]	135	47	182	3:1	0.066	0.79

[a] Resistant lines, AUDPC = 0; susceptible lines, AUDPC > 0

[b] Resistant lines, 0 ≤ AUDPC ≤ 22; susceptible lines, AUDPC > 22

Table 6 Phenotypic classes based on their reaction against stripe rust among the DH lines derived from the Apache × Taldor cross and evaluated under field conditions with three pathotypes

Pathotypes (virulence)	Resistance genes postulated in the phenotypic classes				
	Class A: *Yr7* + *Yr17* or *Yr7* + APR-4B[a] or *Yr7* + *Yr17* + APR-4B 66 lines	Class B: *Yr7* 24 lines	Class C: *Yr17* or *Yr17* + APR-4B 47 lines	Class D: APR-4B 22 lines	Class E: – 22 lines
237E141 (*v1, 2, 3, 4, 6, 9, 11, 25, SU*)	R	R	R	S	S
237E141V17 (*v1, 2, 3, 4, 6, 9,11,17, 25, SU*)	R	R	S	S	S
106E139 (*v2, 3, 4, 7, 11, 25, SU*)	R	S	R	R	S

R resistant lines (AUDPC = 0, except for isolate 106E139, 0 ≤ AUDPC ≤ 22), *S* susceptible lines

[a] APR-4B = *QYr.inra-4B*

Aldeco by Jagger et al. (2011) and corresponding to an APR gene, like *QYr.inra-4B*. This APR gene in Aldeco was associated with a rapid, confined necrotic response, which was not detected in Apache.

A minor QTL, derived from Taldor, was also detected: *QYr.inra-2AL*. This QTL was detected in two trials, 2006/2008, based on the pathotype 237E141V17. The APR QTLs, *QYr.sun-2A* from cv. Kukri (Bariana et al. 2010) and *QYr.inra-2AL*, were detected in the same map interval corresponding to the proximal region of the closely linked marker loci, *Xbarc5* and *Xgwm47*, respectively. Both QTLs are probably race-specific, as described by Bariana et al. (2010), because they were efficient against one single pathotype.

The origins of *Yr7* and *Yr17* found in cv. Apache are not clear because Apache has a complex pedigree. However, Camp Rémy, an ancestor of Apache, carries the *QYr.inra-2BL* ($R^2 = 61$ %) QTL, which is responsible for seedling-stage resistance and may correspond to a cluster of genes, including *Yr7* and *Rsp* (Mallard et al. 2005). The seedling gene *Rsp* could be responsible for the long-term effectiveness (1981–2011) of Camp Rémy seedling resistance against all pathotypes in France. We can also postulate that *Yr7* is present in Camp Rémy, because the resistance of some SSD lines derived from the Camp Rémy × Récital cross was overcome by the French pathotype 106E139 (*v2, 3, 4, 7, 11, 25, SU*), possessing the virulence *V7* and because we know that cv. Récital carries only *Yr6* (de Vallavieille-Pope et al. 1990).

Apache has been grown in France for over 10 years during which conditions conducive for stripe rust epidemics occurred, yet it remains totally resistant. The area dedicated to Apache has been fairly high, 10–24 % of the total area sown with wheat (~5 million ha), which corresponds to 0.5–1 million ha cropped with Apache every year since 2002. It is generally agreed that the use of race-specific resistance against yellow rust should be avoided, but such genes could still be of great help in enhancing and/or protecting durability, if they are used in combination with non-race-specific resistances (Rubiales and Niks 2000). The resistance durability in the cultivars Cappelle Desprez (Johnson 1984), Camp Rémy (Mallard et al. 2005) and Renan (Dedryver et al. 2009), grown in France for many years, is due to a combination of race-specific and non-race-specific major resistance genes with quantitative APR genes for which no stripe rust pathogen has yet evolved the necessary combination of virulence genes. The polygenic nature of the durable Apache resistance is novel, because it is mainly based on a combination of race-specific resistance genes (*Yr7*, *Yr17* and the APR gene *QYr.inra-4B*). So, which hypotheses can be proposed to explain the durability of Apache resistance?

The evolution of pathotypes in Northern Europe has shown on several occasions that the combination of two resistance genes is not sufficient for durable protection of wheat crops. When *Yr9* broke down, cultivars with *Yr9* and *Yr6* were selected, but this resistance was rapidly overcome. Similarly, after *Yr17* broke down, *Yr6* was added to *Yr17* in the same cultivars but it was not long lasting (de Vallavieille-Pope et al. 2012). The pyramiding of three resistance genes, two at the seedling stage and one at the APR stage may explain the efficiency and durability of Apache's resistance. This would emphasize the usefulness of associating more than two resistance genes.

Nevertheless, generalizations in this regard could be quite dangerous. What is true in the case of cv. Apache may not be true when using other combinations of race-specific resistance genes. The durability of the combination of *Yr17*, *Yr7* and the new APR gene found in Apache may be due to a fitness cost of a virulence combination, or a particular context. The cost of virulence was shown for *V4* and *V6* when not useful in the competition of pathotypes inoculated simultaneously on one cultivar (Bahri et al. 2009). The virulence for *Yr17* is common in France but the virulence against *Yr7* has disappeared from the North of France since 1984 when Talent, the cultivar carrying *Yr7*, was no longer grown. The pathotypes *V1, 2, 3, 4, 6, 7, 9, 17, 25*, and *SU*, able to overcome Apache resistance at the seedling stage, have never been established in France (de Vallavieille-Pope et al. 2012), and have not been detected in the UK or in Denmark in the last 10 years (http://www.eurowheat.org). Nevertheless, no information is available on the virulence cost of *V7* and *V17* when used together.

Another explanation for the durability of cv. Apache resistance may be that the conditions were not favourable for a spread of pathotypes that overcome cv. Apache resistance. Cv. Apache has been widely grown in France for nearly 10 years, but due to the earliness of this cultivar it is not used in Northern Europe. Studies concerning the dynamics of new pathotype emergence and spread in the North of France show that all the new pathotypes that have appeared in France at one time point originated from the United Kingdom (UK) or Denmark (DK). The pathotypes carrying *V17* first appeared in the UK and in DK in 1993 and 4 years later in France and Germany (Bayles et al. 2000). Wheat cultivars with *Yr17* resistance first became popular in UK and DK in 1993/1994 and over the next few years increased rapidly to around 35 % (mainly cv. Brigadier, carrying *Yr17*) of the UK acreage and 55 % (mainly cv. Hussar, carrying *Yr9*, *Yr17* and one unknown gene, Hovmøller 2007) of the acreage of DK. Over the next 3 years, virulence for *Yr17* built up to nearly 100 % frequency in UK, and the same frequency was observed in 1997 and 1998 in DK (Bayles et al. 2000). Despite the relatively low selection pressure from *Yr17* cultivars in

France and Germany, virulence was detected in both countries in 1997 and reached frequencies of over 70 % by 1999 (Bayles et al. 2000). Similarly, the pathotypes *V32* appeared earlier in the UK and DK than in France (http://www.eurowheat.org). As cv. Apache could not be grown in these countries, pathotypes with a combination of virulences overcoming Apache resistance were not selected. If this hypothesis is true, this means that the durability of cv. Apache resistance is the result of fortuitous circumstances. Nevertheless, a major lesson that we can draw from the Apache case study may be that a combination of two or three race-specific resistance genes can be durable, provided that it is properly managed at a continental level by carefully taking into account the dynamics of the pathogen population. Especially, in the case of stripe rust, the pathogen population occurring in France and Germany in a given year is mainly influenced by the selection pressure that shaped the pathogen populations of UK and DK a few years before. It is thus strongly advisable to use different combinations of resistance genes in France and Germany compared to UK and DK. The increasing number of molecular markers linked to already known resistance genes and QTLs, as well as an increasing number of genetic characterization of the resistances used in breeding programmes will help to achieve this objective. This large-scale approach for resistance gene deployment has been advised in the USA for stem rust with different genes between southern USA and Canada, taking the prevailing winds into consideration (Wolfe and Knott 1982).

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