Eur J Plant Pathol (2011) 130:559–575
DOI 10.1007/s10658-011-9778-2

Diagnostic value of molecular markers for *Lr* genes and characterization of leaf rust resistance of German winter wheat cultivars with regard to the stability of vertical resistance

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Accepted: 18 March 2011 / Published online: 2 April 2011

Abstract Breeding for resistance is an efficient strategy to manage wheat leaf rust caused by *Puccinia triticina* f. sp. *tritici*. However, a prerequisite for the directed use of *Lr* genes in breeding and the detection of new races virulent to these *Lr* genes is a detailed knowledge on *Lr* genes present in wheat cultivars. Therefore, respective molecular markers for 18 *Lr* genes were tested for specificity and used to determine *Lr* genes in 115 wheat cultivars. Results obtained were compared to available pedigree data. Using respective molecular markers, genes *Lr1*, *Lr10*, *Lr26*, *Lr34* and *Lr37* were detected, but data were not always in accordance with pedigree data. However, leaf rust scoring data of field trials confirmed the reliability of DNA markers. These reliable marker data facilitated the analyses of the development of virulent leaf rust races from 2002 to 2009 based on released cultivars. A sudden change from low infection rates to susceptibility was observed for *Lr1*, *Lr3, Lr10, Lr13, Lr14, Lr16, Lr26 and Lr37* since 2006. Cultivars carrying several leaf rust resistance genes showed no significant shift to susceptibility except one cultivar which revealed an increasing infection rate at a low level. In summary, it turned out that pedigree data are often not reliable and a detection of *Lr* genes by diagnostic markers is fundamental to combine *Lr* genes in cultivars for a durable resistance against leaf rust, and to conduct reliable surveys based on released cultivars, instead of 'Thatcher' NILs.

Keywords Epidemiology · Molecular marker · *Puccinia triticina* pathotypes · Resistance · *Lr* genes

Abbreviations

CAPS	Cleaved amplified polymorphic DNA
cv.	Cultivar
Lr	Leaf rust resistance
NIL	Near-isogenic lines
SCAR	Sequence characterized amplified region
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeats
STR	Short tandem repeat
STS	Sequence tagged site
ANOVA	Analysis of variance

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Introduction

Leaf rust of wheat caused by *Puccinia triticina* is one of the most important diseases of wheat resulting in high yield losses and reduced grain quality (Cloutier

et al. 2007). Breeding of resistant wheat cultivars is the most cost effective and environmentally sound strategy to prevent these losses. Therefore, many *Lr* genes were introgressed into wheat mainly derived from related species (reviewed by Bolton et al. 2008). Different molecular marker techniques allow a large-scale, time-adequate and cost-effective detection of resistance genes against leaf rust (Stępień et al. 2003). The validation of molecular markers in different genetic backgrounds is a prerequisite for applying them in marker assisted selection procedures (Błaszczyk et al. 2008). Although numerous molecular markers are described for leaf rust resistance loci by different authors, little information is available about their practical use in wheat breeding (Stępień et al. 2003). For the following *Lr* resistance genes, PCR based markers (SNPs, SSRs, STS, SCARs, CAPSs) were developed: *Lr1, Lr9, Lr10, Lr13, LR16. Lr19, Lr20, Lr21/Lr40, Lr24, Lr25, Lr26, Lr28, Lr29, Lr34, Lr35, Lr37, Lr39/41, Lr46, Lr47, Lr50, Lr51, Lr52* (Tyrka et al. 2004; Chełkowski et al. 2003; Schachermayr et al. 1997; Błaszczyk et al. 2008; Huang and Gill 2001; Mago et al. 2002; Lagudah et al. 2009; Gold et al. 1999; Helguera et al. 2003, 2005; Raupp et al. 2001; Obert et al. 2005). All molecular markers for the above-mentioned *Lr* genes are described as closely linked with the exception of markers for *Lr13*, *Lr16*, *Lr46* and *Lr50* for which the genetic distance between marker and gene is more than 3 cM. These markers were therefore not included in our investigations. The specificity of molecular markers for *Lr9*, *Lr10*, *Lr19*, *Lr24* and *Lr29* was confirmed in different studies (Chełkowski et al. 2003, Błaszczyk et al. 2008) in a wide range of genetic backgrounds. Most of the *Lr* resistance loci confer race specific resistance leading to a hypersensitive reaction and/or lignification of cell walls (Bolton et al. 2008). Due to the high level of virulence variation in different rust isolates, race specific resistance is in general not durable and the breakdown of resistance has been described for *Lr2, Lr3,Lr9*, *Lr11*, *Lr18*,Lr24, *Lr26* (Kolmer 2005). Non-race-specific resistance is conferred by only two *Lr* genes, i.e. *Lr34* (Krattinger et al. 2009) and *Lr46* (Singh et al. 1998) which are not associated with a hypersensitive reaction. The isolation and molecular characterization of *Lr1* (Cloutier et al. 2007), *Lr10* (Loutre et al. 2009) and *Lr21* (Huang and Gill 2001), as race specific coiled coil- nucleotide-binding site-leucine-rich repeats coding genes, explains the higher durability of *Lr34* which is a race unspecific adenosine triphosphate–binding cassette-transporter (Krattinger et al. 2009).

Analysing leaf rust populations in Europe revealed a high complexity of virulence in pathotypes (Park et al. 2001) and a correlation between the occurrence of resistance-breaking isolates and the area cultivated with varieties carrying respective resistance genes; e.g. in 1995 leaf rust pathotypes virulent on *Lr2*, *Lr11*, *Lr17, Lr26* were detected with high frequencies while at the same time cultivars carrying these resistance genes were grown on a large acreage cv. 'Contra' which carries *Lr17*, area sown 8,4%, cv. 'Toronto' carrying *Lr26*, area sown 7% (Park et al. 2001). Due to such events, scoring of leaf rust infection over time is necessary. For this purpose, near isogenic lines (NILs) carrying corresponding *Lr* genes in the genetic background of the susceptible cv. 'Thatcher' are best suited. These NILs were created by several cycles of backcrossing.

Therefore, the aim of this study was: (a) to evaluate molecular markers for leaf rust resistance for their diagnostic value with regard to their robustness and validity in different genetic backgrounds, (b) to screen a set of 115 German winter wheat cultivars listed in the "Descriptive Variety List" of the Federal Seed Board of Germany (http://www.bundessortenamt.de/internet30/index.php?id=23) with these markers in order to get information on the *Lr* genes present in German cultivars, (c) to detect the occurrence of new *Lr* resistance breaking leaf rust pathotypes with different virulence by annual analyses of NILs and cultivars in field trials, and (d) to get information on the reliability of pedigree data compared to data obtained by marker analyses.

Material and methods

Plant material

The 115 German winter wheat cultivars investigated in this study are listed in Table 1 and were provided by D. Rentel (Bundessortenamt, Hannover, Germany). The specificity of molecular markers was analysed on NILs of cv. 'Thatcher' carrying *Lr1*, *Lr3*, *Lr9*, *Lr10*, *Lr13*, *Lr16*, *Lr18*, *Lr19*, *Lr20*, *Lr21/Lr40*, *Lr24*, *Lr25*, *Lr26*, *Lr28*, *Lr29*, *Lr34*, *Lr35*, *Lr37* and *Lr52*. 'Thatcher' NILs were kindly provided by Prof. A. Mesterhàzy (Cereal Res. Inst., Szeged, Hungary). There were no NILs available for *Lr39/Lr41*, *Lr47*

Table 1 Pedigrees of investigated cultivars, deduced *Lr* genes, results of marker analysis and infection ratings of monitoring in 2006 and 2007. Expected PCR product indicating an Lr gene are shown by "+". Absence of PCR products or unspecific fragments not diagnostic for the respective Lr gene are displayed by "–". The results for genes *Lr9*, *Lr19*, *Lr20*, *Lr21/Lr40*, *Lr24*, *Lr25*, *Lr28*, *Lr29*, *Lr35*, *Lr39/Lr41*, *Lr47*, *Lr51*, *Lr52* are not shown as PCR products could not be detected in any of the tested cultivars. Results of monitoring show the infection ratings in relation to the highly susceptible standard cv. Borenos. Significant differences between 2006 and 2007 are marked by asterisks. An "x" indicates that pedigree data are known but confidential data of breeders

Wheat variety	Pedigree	Reference	Rating related to cv Borenos (%)			Possible Lr genes	Molecular marker analyses				
			2006	2007	sign.	deduced from pedigree	Lr1	Lr10	Lr26	Lr34	Lr37
Actros	X	confidential data	50.0±11.5	45.0±12.9		*Lr3, Lr10, Lr13, Lr16, Lr26, Lr37*	–	–	–	–	+
Akratos	X	Goyeau and Lannou 2010	42.5±12.6	75.0±10.0	*	*Lr13*	–	–	–	–	–
Akteur	Stamm 87–308/Astron//Astron	European Wheat Database EWDB	45.0±17.3	50.0±11.5		unknown	–	–	–	–	–
Akzento	X	European Wheat Database EWDB	20.0±8.2	36.7±5.8	*	unknown	–	+	–	–	+
Alidos	Arkos/Hadmerslebener–00914–76	European Wheat Database EWDB	25.0±11.5	60.0±0.0	*	*Lr14*	–	–	–	–	–
Alitis	X	confidential data	42.5±12.6	35.0±5.8		*Lr13, Lr14, Lr26*	–	–	–	–	–
Altos	X	confidential data	42.5±12.6	40.0±0.0		*Lr10, Lr13, Lr14*	–	–	–	–	–
Amply	X	confidential data	45.5±10.5	85.0±19.1	*	*Lr37*	–	–	–	–	+
Anthus	X	confidential data	50.0±11.5	40.0±8.2		*Lr10, Lr13*	–	–	–	–	+
Aristos	((RPB 49.75 x Stamm aus Huntsman) x Glaucus) x Urban	European Wheat Database EWDB	27.5±9.6	60.0±0.0	*	*Lr13*, *Lr14*, *Lr26*	–	–	–	–	–
Arminius	X	confidential data	40.0±11.6	85.0±19.1	*	unknown	–	–	–	–	–
Asketis	X	confidential data	20.0±8.2	60.0±8.2	*	*Lr13, Lr14, Lr26*	–	–	–	–	–
Aspirant	X	confidential data	30.0±11.5	57.5±12.6	*	unknown	–	–	–	–	–
Batis	RPB–4945//Maris–Huntsman/Glanens/3/Urban	European Wheat Database EWDB	11.5±6.0	30.0±8.2	*	*Lr13*, *Lr14*, *Lr26*	–	–	–	–	–
Biscay	CPB.79/Hussar	Goyeau and Lannou 2010	9.0±2.0	27.5±9.6	*	*Lr10*, *Lr13*, *Lr37*	–	+	–	–	+
Bold	X	convidential data	71.3±21.7	58.9±14.7		*Lr16*, *Lr37*	–	–	–	–	–
Boomer	Quilafen/Kill	European Wheat Database EWDB	32.5±9.6	40.0±11.5		*Lr13*	–	–	–	–	+
Borneo	Andros/Urban	Varshney and Altpeter 2001	52.5±9.6	83.8±11.1	*	*Lr13*, *Lr14*, *Lr26*	–	–	–	–	–
Brilliant	Hadm. 22779–87/Greif//Tarso	European Wheat Database EWDB	20.0±8.2	35.0±5.8	*	*Lr3*, *Lr10*, *Lr13*, *Lr17*, *Lr26*	–	+	+	–	–
Bussard	Kranich/Maris–Huntsman//Monopol	European Wheat Database EWDB	70.0±20.0	90.0±11.5		*Lr10*, *Lr13*, *Lr14*	–	–	–	–	–
Buteo	LP 4285.90/LP 3273.87//Pegassos	European Wheat Database EWDB	32.5±9.6	55.0±10.0	*	*Lr13*, *Lr14*, *Lr26*	–	–	–	–	–
Campari	X	confidential data	42.5±12.6	37.5±9.6		*Lr10*, *Lr13*, *Lr26*	–	+	+	–	–
Capnor	X	Goyeau and Lannou 2010	32.5±9.6	37.5±9.6		*Lr14*, *Lr37*	–	–	–	–	+

Table 1 (continued)

Wheat variety	Pedigree	Reference	Rating related to cv Borenos (%)			Possible Lr genes	Molecular marker analyses				
			2006	2007	sign.	deduced from pedigree	Lr1	Lr10	Lr26	Lr34	Lr37
Cardos	Cappelle–Desprez//Taras/ Hadmerslebener–230–60	Goyeau and Lannou 2010	20.0±8.2	35.0±5.8	*	*Lr13*, *Lr37*	–	–	–	–	+
Carenius	X	confidential data	cv. released after 2007		*Lr1, Lr3, Lr26, Lr37*	–	+	+		+	
Centrum	Hussar/Konsul//Lambros	Badea et al. 2008	20.0±8.2	37.5±5.0	*	*Lr26*, *Lr37*	–	+	–	–	+
Certo	brl/Rendezvous//Marabu	Varshney and Altpeter 2001	32.5±9.6	45.0±10.0		*Lr26*, *Lr37*	–	–	+	–	+
Cetus	X	confidential data	6.0±4.6	3.0±3.5		*Lr3, Lr10, Lr13, Lr14, Lr26, Lr37*	–	–	–	–	+
Champion	X	European Wheat Database EWDB	22.5±9.6	25.0±10.0		*Lr13*	–	–	+	–	–
Compliment	X	European Wheat Database EWDB	55.0±10.0	120.0±0.0	*	unknown	–	–	–	–	–
Convent	X	European Wheat Database EWDB	102.5±30.7	78.0±7.6		*Lr10, Lr13*	–	–	–	–	–
Cortez	X	confidential data	40.0±11.5	55.0±10.0		*Lr1*	+	–	–	–	–
Creativ	X	confidential data	37.5±17.1	45.0±10.0		*Lr10, Lr13, Lr26*	–	+	–	–	+
Darwin	X	confidential data	32.5±9.6	50.0±11.5	*	*Lr10, Lr13, Lr14*	–	+	–	–	–
Dekan	brl/brl//Greif	Varshney and Altpeter 2001	32.5±9.6	72.5±18.9	*	*Lr10*, *Lr13*	–	+	–	–	–
Dream	St. SCHW 97–80/Orestis	Schmolke et al. 2005	77.5±15.0	90.0±11.5		*Lr37*	–	–	–	–	–
Drifter	Ronos/Estica	European Wheat Database EWDB	47.5±15.0	70.0±20.0		*Lr13*, *Lr14*, *Lr26*	–	–	–	–	–
Elegant	X	confidential data	22.5±9.6	32.5±12.6		*Lr1, Lr10, Lr13 Lr26, Lr37*	–	+	+	–	+
Empire	Disponent/Kronjuwel// Monopol/Kurier	Badea et al. 2008	32.5±9.6	105.0±10.0	*	*Lr26*	–	–	–	–	–
Estica	Arminda/Virtue	Varshney and Altpeter 2001	22.5±5.0	25.0±10.0		*Lr13*, *Lr14*	–	–	–	–	–
Excellenz	X	European Wheat Database EWDB	32.5±9.6	27.5±9.6		*Lr1, Lr10, Lr13, Lr26, Lr37*	–	+	+	–	+
Frodin	X	confidential data	32.5±9.6	47.5±15.0		*Lr10, Lr13, Lr14, Lr17, Lr26, Lr37*	–	+	–	–	+
Gaston	X	confidential data	36.4±14.8	37.5±5.0		*Lr10, Lr13, Lr14, Lr17, Lr26, Lr37*	–	–	–	–	+
Greif	Maris–Hobbit/2*Carimulti	European Wheat Database EWDB	20.0±8.2	45.0±10.0	*	*Lr10*, *Lr13*	–	+	–	–	–
Habicht	brl/Sperber//Greif	Varshney and Altpeter 2001	32.5±11.5	70.0±11.5	*	*Lr10*, *Lr13*	–	–	–	–	–
Hermann	Nic90–3390A/Xanthos	Badea et al. 2008	32.5±9.6	30.0±8.2		*Lr13*, *Lr14*	–	+	–	–	+
Heroldo	Tambor/Greif//Kris	European Wheat Database EWDB	32.5±9.6	50.0±11.5		*Lr10*, *Lr13*	–	+	–	–	+
Hybnos 1	X	European Wheat Database EWDB	47.5±15	50.0±11.5		*Lr10, Lr13, Lr14, Lr37*	–	+	–	–	+

Hybnos 2B	X	confidential data	82.5±17.1	100.0±0.0		*Lr13, Lr14, Lr37*	–	–	–	–	+
Hybred	HybrideEuro97–15/Piko	Badea et al. 2008	32.5±9.6	120±23.1	*	*Lr10*, *Lr13*, *Lr14*, *Lr37*	–	+	–	–	+
Idol	X	European Wheat Database EWDB	76.7±6.7	85.0±19.1		*Lr14, Lr37*	–	–	–	–	–
Impression	X	confidential data	cv. released after 2007		*Lr13, Lr26, Lr37*	–	–	–		+	
Koch	X	confidential data	0.0±0.0	0.0±0.0		unknown	–	–	–	–	–
Korund	X	European Wheat Database EWDB	80.0±23.1	65.0±10.0		*Lr13,Lr26*	–	–	–	–	–
Kris	Hereward/Rendezvous//Torfrida	European Wheat Database EWDB	32.5±9.6	55.0±10.0	*	*Lr10*, *Lr13*, *Lr37*	–	+	–	–	+
Lahertis	X	confidential data	7.0±3.8	10.3±6.8		*Lr13, Lr14, Lr26*	–	–	+	–	–
Leiffer	X	confidential data	40.0±0.0	35.0±5.7		*Lr10, Lr37*	–	–	–	–	+
Limes	X	Goyeau and Lannou 2010	40.9±15.7	47.5±15.0		*Lr1*, *Lr10*	+	+	–	–	–
Lucius	X	confidential data	47.5±15.0	85.0±5.8	*	*Lr13*	–	–	–	–	–
Madrid	X	confidential data	45.5±10.5	55.0±10.0		*Lr13, Lr14*	+	–	–	–	–
Magister	Ebi/W 86151	European Wheat Database EWDB	47.5±15.0	32.5±9.6		*Lr26*	–	–	–	–	+
Magnus	X	confidential data	32.5±9.6	37.5±17.1		*Lr13, Lr14*	–	–	–	–	–
Manager	X	Goyeau and Lannou 2010	32.5±9.6	50.0±11.4	*	*Lr37*	–	–	–	–	+
Mandub	X	confidential data	0.0±0.0	2.5±5.0		unknown	–	–	–	–	–
Manhattan	X	European Wheat Database EWDB	22.5±5.7	50.0±11.5	*	*Lr13, Lr14*	–	–	–	–	–
Maverick	X	confidential data	32.5±9.6	70.0±20.0	*	*Lr10, Lr37*	–	+	–	–	+
Meteor	X	confidential data	cv. released after 2007		*Lr3, Lr10, Lr13, Lr17, Lr26, Lr37*	–	–	+	–	+	
Milvus	X	confidential data	32.5±9.6	50.0±11.5		*Lr10, Lr13, Lr14, Lr17, Lr26*	–	–	–	–	–
Mirage	X	confidential data	cv. released after 2007		Unknown	–	+	+	–	–	
Moldau	Hana/Mercia	Badea et al. 2008	37.5±17.1	85.0±19.2	*	*Lr3*, *Lr13*, *Lr14*, *Lr16*	–	+	–	–	–
Monopol	Pantus/Admiral	European Wheat Database EWDB	55.0±10.0	70.0±20.0		*Lr10*	–	–	–	–	–
Motiv	X	European Wheat Database EWDB	70.0±11.5	80.0±23.1		*Lr10, Lr13*	–	+	–	–	–
Mulan	Ronus/Estica//Maverick	European Wheat Database EWDB	cv. released after 2007		*Lr10*, *Lr13*, *Lr14*, *Lr37*	–	+	–	–	+	
Naturastar	Severin.Mo.Ht/Mission//Ares.Urban	Badea et al. 2008	90.0±20.0	82.5±17.1		*Lr13*, *Lr14*, *Lr26*	–	–	–	–	–
Noah	X	confidential data	29.5±4.5	47.5±15.0		*Lr37*	–	–	–	–	+
Pegassos	RPB–49–75/3/Maris–Huntsman/Glaucus//Urban	European Wheat Database EWDB	14.0±7.1	70.0±20.0	*	*Lr13*, *Lr14*, *Lr26*	–	–	–	–	–
Petrus	Nimbus/Vuka//Falke/3/Benno/Caribo//Kormoran9/Kronjuwel	Badea et al. 2008	22.5±9.6	45.0±10.0	*	*Lr26*	–	–	+	–	–

Table 1 (continued)

Wheat variety	Pedigree	Reference	Rating related to cv Borenos (%)			Possible Lr genes	Molecular marker analyses				
			2006	2007	sign.	deduced from pedigree	Lr1	Lr10	Lr26	Lr34	Lr37
Piko	CWW–3319.5/3/Kraka//Maris–Huntsman/Fruhgold	European Wheat Database EWDB	37.5±17.1	60.0±0.0	*	*Lr10*, *Lr13*, *Lr14*, *Lr37*	–	+	–	–	+
Potenzial	X	confidential data	cv. released after 2007		*Lr10, Lr13, Lr37*	–	+	–	–	+	
Privileg	X	confidential data	37.5±17.1	32.5±9.6		*Lr26*	–	–	–	–	+
Punch	X	confidential data	42.5±5.0	50.0±0.0	*	*Lr10, Lr37*	–	+	–	–	+
Qualibo	X	confidential data	40.0±8.2	52.5±5.0		*Lr10, Lr13, Lr26*	–	+	–	–	+
Quebon	X	Goyeau and Lannou 2010	55.0±10.0	60.0±0.0		*Lr10*, *Lr13*, *Lr37*	–	+	–	–	+
Ramiro	Mironovskaya–808//Bezostaya–1/Erythrospmermum–1526	European Wheat Database EWDB	discontinued before 2006		*Lr3*, *Lr10*, *Lr34*	–	–	–	+	–	
Ranger	X	confidential data	37.5±12.5	55.0±10.0		*Lr10, Lr26*	–	+	+	–	–
Reaper	X	confidential data	discontinued before 2006		*Lr10, Lr13, Lr17, Lr26, Lr37*	–	+	–	–	+	
Redford	X	European Wheat Database EWDB	100.0±0.0	90.0±8.2		*Lr10, Lr13*	–	–	–	–	–
Renan	Mironovskaya–808/Maris–Huntsman//VPM/Moisson/3/Courtot	Goyeau and Lannou 2010	discontinued before 2006		*Lr13, Lr37*	–	–	–	–	+	
Ritmo	Hobbit/Line–1320//Wizard/3/Marksman/Virtue	Goyeau and Lannou 2010	55.0±10.0	97.5±31.0	*	*Lr13*	–	–	–	–	–
Romanus	Estica/Urban	Badea et al. 2008	37.5±17.1	45.0±12.9		*Lr13*, *Lr14*, *Lr26*	–	–	–	–	–
Semper	X	confidential data	10.0±5.7	82.5±17.1	*	*Lr3*	–	–	–	–	–
Skagen	X	confidential data	cv. released after 2007		*Lr10, Lr13, Lr17*	–	–	–	–	–	
Skater	X	European Wheat Database EWDB	47.5±15.0	55.0±5.8		unknown	–	–	–	–	–
Sobi	1553 fl32/1730d53//Transit	Badea et al. 2008	37.5±17.1	27.5±9.6		*Lr13*	–	–	+	–	–
Sokrates	Xanthos/Stamm	Badea etal. 2008	97.5±5.0	105.0±10.0		*Lr13*, *Lr14*	–	–	–	–	–
Solitär	Flair/Piko	Badea et al. 2008	40.0±16.3	55.0±10.0		*Lr10*, *Lr13*, *Lr37*	–	–	–	–	+
Striker	X	confidential data	32.5±9.6	22.5±5.0		*Lr26*	–	+	–	–	+
SW Maxi	X	confidential data	32.5±9.6	55.0±10.0	*	*Lr14*	–	–	–	–	–
SW Topper	X	confidential data	27.5±5.0	55.0±10.0	*	*Lr13*	–	–	–	–	–
Terrier	X	European Wheat Database EWDB	90.0±20.0	80.0±23.1		*Lr10, Lr13, Lr26*	–	–	–	–	–
Tommi	NORD 92–147//Astron/4442	European Wheat Database EWDB	27.3±7.4	60.0±0.0	*	*Lr37*	–	–	–	–	+
Toni	X	European Wheat Database EWDB	discontinued before 2006		*Lr13, Lr14, Lr37*	–	–	–	–	+	
Toras	Taras/Stamm//Herevard/3/Tarso	Badea et al. 2008	47.5±15.0	45.0±10.0		*Lr3*, *Lr10*, *Lr13*, *Lr17*, *Lr26*	–	+	–	–	–
Transit	Urban/Apollo	Varshney and Altpeter 2001	19.0±9.9	30.0±11.5		*Lr13*, *Lr26*	–	–	+	–	–

Travix	Rialto/Lynx	Badea et al. 2008	22.7±9.1	35.0±10.0	*Lr1, Lr10, Lr13, Lr26, Lr37*	+	+	+	–	+	
Trend	Greif/Ibis	Badea et al. 2008	129.2±27.6	74.4±6.0	*Lr10, Lr13*	–	–	–	–	–	
Tuareg	X	confidential data	27.5±9.6	47.5±15.0	*Lr37*	–	–	–	–	+	
Tukan	Kranich/Caribo	European Wheat Database EWDB	27.5±5.0	40.0±11.5	unknown	–	–	–	–	+	
Tulsa	X	confidential data	0.0±0.0	0.0±0.0	unknown	–	–	–	–	–	
Türkis	X	European Wheat Database EWDB	38.6±11.4	70.0±20.0	*Lr37*	–	–	–	–	+	
Vergas	CWW5230.1/Kronjuwel// Monopol	Badea et al. 2008	36.6±14.8	50.0±11.5	*Lr26*	–	–	+	–	–	
Wenga	X	confidential data	45.5±10.5	42.5±5.0	*Lr14, Lr26, Lr34*	–	–	+	+	–	
Winnetou	Apollo/F 1830	European Wheat Database EWDB	37.5±5.0	40.0±0.0	*Lr13, Lr26*	–	+	+	–	–	
Zentos	Hadmerslebener–39687–76/ Compal	Varshney and Altpeter 2001	90.0±20.0	85.0±19.0	unknown	–	–	–	–	–	
Zobel	X	confidential data	cv. released after 2007		*Lr3, Lr10, Lr13, Lr26, Lr37*	–	–	–	–	+	

and *Lr51*. In this case, markers were tested on wheat accessions known to possess the corresponding genes (*Lr39/41*-PI 592728, *Lr47*-PI 603918, *Lr51*/F.7.3). In addition to the 'Thatcher' lines, respective markers were analysed on genotypes reported in the literature to carry respective *Lr* genes (Table 2).

Field trials, rating of leaf rust symptoms and statistical analysis

For virulence analysis, leaf rust symptoms were scored in different field trials carried out from 2002 to 2005 at Aschersleben (Saxony-Anhalt, Germany, 113 m above sea level) and from 2006 to 2009 at Quedlinburg (Saxony-Anhalt, Germany, 125 m above sea level). The field in Aschersleben is located at 51°756777` to 51°761439` North, 11°427804` to 11°430802`East and 51°771186` to 51°770801` North, 11°142712` to 11.149557 East, and in Quedlinburg from 51°771438` to 51°769327` North and from 11°143141` to 11°149685` East. The two locations are located about 20 km apart in one of the main wheat producing areas in Germany. At Quedlinburg the long-term average of temperature from 1980 to 2010 was 8.9°C, and the yearly sum of precipitation was 497 mm. At Aschersleben it was 8.8°C and 476 mm, repectively. Both sides are characterized by a loamy loess with 90 to 95 points according to the German soil taxonomy scale. Infection conditions and incidence of leaf rust were comparable at both sides as scores observed for the cultivar 'Borenos' (standard) were similar. Detailed data are given in the results section.

Field trials of the NILs including the standard cv. 'Thatcher' were carried out from 2002 to 2009. In the direct vicinity of these trials, the trials comprising the cultivars carrying respective resistance genes (Table 1) were conducted including the standard cv 'Borenos'. These field trials were conducted from 2004 to 2008. Results of infection ratings from the years 2006 and 2007 are displayed in Table 1. Both trials were arranged in a fully randomised block design with four replications (plot size 1.2 m x 0.9 m). Scoring of leaf rust symptoms was conducted according to James (1971) at the beginning of flowering by screening flag leaves of 20 plants per plot. To take into account different infection levels between years, scoring of the cultivar 'Thatcher' (without any *Lr* gene) was set to 100 and the scorings of NILs carrying different *Lr* loci were calculated relative to 'Thatcher'. In the

Table 2 Verification of *Lr* genes postulated by pedigrre data by marker analysis. Fragment sizes indicative for a specific *Lr* gene are indicatated by "+", no PCR fragments or fragments of different size are indicated by "–"

Accession	Gene	Marker											
		Lr21						*Lr39*					
		Lr1	*Lr20*	*Lr40*	*Lr25*	*Lr26*	*Lr34*	*Lr35*	*Lr37*	*Lr41*	*Lr47*	*Lr51*	*Lr52*
Citr 15237	*Lr1*	+	–	–	–	–	–	–	–	–	–	–	–
Citr 15236	*Lr3*	–	–	–	–	–	–	–	–	–	–	–	–
Citr 17905	*Lr9*, *Lr24*	–	–	–	–	–	–	–	–	–	–	–	–
Citr 13775	*Lr13*	–	–	–	–	–	–	–	–	–	–	–	–
Citr 15239	*Lr16*	–	–	–	–	–	–	–	–	–	–	–	–
Citr 15242	*Lr18*	–	–	–	–	–	–	–	–	–	–	–	–
Citr 14048	*Lr19*	–	–	–	–	–	–	–	–	–	–	–	–
Sunnan	*Lr19*	–	–	–	–	–	–	–	–	–	–	–	–
PI 93988	*Lr20*	–	+	–	–	–	–	–	–	–	–	–	–
Citr 17755	*Lr21=40*	–	–	+	–	–	–	–	–	–	–	–	–
KS86WGRC02	*Lr21=40*	–	–	+	–	–	–	–	–	–	–	–	+
PI 519472	*Lr21=40*	–	–	+	–	–	–	–	–	–	–	–	–
PI 520526	*Lr21=40*	–	–	+	–	–	–	–	–	–	–	–	+
Citr 17474	*Lr24*	–	–	–	–	–	–	–	–	–	–	–	–
Citr 14189	*Lr25*	–	–	–	–	–	–	–	–	–	–	–	–
Citr 17927	*Lr25*	–	–	–	–	–	–	–	–	–	–	–	+
PI 636140	*Lr25*	–	–	–	+	–	–	–	–	–	–	–	+
Albrecht	*Lr26*	–	–	–	–	+	–	–	–	–	–	–	+
Apollo	*Lr26*	–	–	–	–	–	–	–	–	–	–	–	–
Arber	*Lr26*	–	–	–	–	+	–	–	–	–	–	–	–
Disponent	*Lr26*	–	–	–	–	+	–	–	–	–	–	–	+
PI 418566	*Lr26*	–	–	–	–	+	–	–	–	–	–	–	+
PI 518799	*Lr26*	–	–	–	–	+	–	–	–	–	–	–	–
PI 520264	*Lr26*	–	–	–	–	+	–	–	–	–	–	–	–
Chinese Spring	*Lr34*	–	–	–	–	–	+	–	–	–	–	–	–
PI 442901	*Lr34*	–	–	–	–	–	+	–	–	–	–	–	–
PI 600683	*Lr35*	–	–	–	–	–	–	+	–	–	–	–	–
PI 638742	*Lr37*	–	–	–	–	–	–	–	+	–	–	–	–
PI 592728	*Lr39/41*	–	–	–	–	–	–	–	–	+	–	–	–
KS90WGRC10	*Lr39/41*	–	–	–	–	–	–	–	–	+	–	–	–
Pavon 76	*Lr46, Lr1*	+	–	–	–	–	–	–	–	–	–	–	–
PI 603918	*Lr47*	–	–	–	–	–	–	–	–	–	+	–	–
PI 604220	*Lr47*	–	–	–	–	–	–	–	–	–	+	–	+
KSWGRC36	*Lr50*	–	–	–	–	–	–	–	–	–	–	–	–
F.7.3	*Lr51*	–	–	–	–	–	–	–	–	–	–	+	–
PI 289824	*Lr52b*	–	–	–	–	–	–	–	–	–	–	–	+

same way cultivar 'Borenos' was taken as a standard for the scoring of the cultivars in field trials. The scoring was based on the percentage leave area infected. The different types of reaction to a leaf rust infection were not analyzed due to the occurrence of leaf rust independent reactions on leaves caused by biotic and abiotic stress factors, respectively. In order to determine whether differences detected between years and cultivars/lines were significant, one way analysis of variance (ANOVA) was performed using the program SAS (version 9.1, SAS Institute, Cary, NC, USA).

DNA extraction

DNA was isolated from 300 mg leaves of 14-day-old seedlings in plastic bags according to the modified cetyl- trimethylammonium-bromide preparation method described by Stein et al. (2001).

PCR amplification

Markers used for the different *Lr* genes are listed in Table 3.

The PCR reactions were set up with the recommended protocol from published data for each marker in a 20 µl reaction volume containing 100 ng DNA. For robust amplification of described PCR products some PCR conditions were optimised as shown in Table 4. *Taq* DNA polymerase (Qiagen, Hilden, Germany) and AmpliTaq Gold polymerase (Applied Biosystems, Darmstadt) were used. Amplification products were separated on 1.5% agarose gel (Applichem, Darmstadt, Germany) or on 3.0% NuSieve 3:1 (Biozym, Hamburg, Germany) agarose gel (*Lr21* marker) in 1 x buffer consisting 0.089 M Tris, 0.089 M boric acid and 0.002 M EDTA (TBE buffer) at 80 V for 2 h, stained with ethidium bromide and visualised on a digital gel documentation system Gel Doc XR Universal Hood II by using the software package Quantity One ver. 4.6.3 (Bio-Rad, München, Germany).

The SNP for *Lr1* was detected using the SNP Primer Extension Kit (Beckman Coulter, Krefeld, Germany) on a CEQ™ 8000 Genetic Analysis System according to Tyrka et al. (2004).

Results

Specificity of DNA markers for Lr genes

The markers for *Lr1*, *Lr20*, *Lr21/Lr40*, *Lr25*, *Lr26*, *Lr34*, *Lr35*, *Lr37*, *Lr39/41*, *Lr47*, *Lr51* and *Lr52*

Table 3 Molecular markers applied for the detection of *Lr* genes

Gene	Marker type	Marker name	References
Lr1	SNP	STS-29 F/275R Lr1-89 F	Tyrka et al. 2004
Lr9	STS	J13-1/J13-2	Chełkowski et al. 2003
Lr10	STS	Lr10F/Lr10R	Schachermayr et al. 1997
Lr19	STS	STSLr19130	Chełkowski et al. 2003
Lr20	STS	STS638	Błaszczyk et al. 2008
Lr21/Lr40	STS	KSUD14	Huang and Gill. 2001
Lr24	STS	J09/1-J09/2	Chełkowski et al. 2003
Lr25	SCAR	Lr25-F20/-R19	Błaszczyk et al. 2008
Lr26	STS	iag95	Mago et al. 2002
Lr28	STS	SCS421570	Błaszczyk et al. 2008
Lr29	SCAR	Lr29-F24/-R24	Błaszczyk et al. 2008
Lr34	SSR	cssfr5	Lagudah et al. 2009
Lr35	SCAR	SR39-F/-R	Gold et al. 1999
Lr37	STS	Ventriup/LN2	Helguera et al. 2003
Lr39/Lr41	SSR	Xgdm35	Raupp et al. 2001
Lr47	STS	PS10-L/-R	Chełkowski et al. 2003
Lr51	CAPS	S30-13 L/AGA7-759R	Helguera et al. 2005
Lr52	STS	TXW200	Obert et al. 2005

Table 4 PCR mixture and conditions for detecting *Lr* genes as well as specific fragment sizes

Gene	Marker	PCR Mix	PCR amplification conditions	estimated base pairs
Lr9	J13-1/J13-2	0.4 mM dNTP, 0.5 μM each Primer, 3.0 mM $MgCl_2$, 0.8 U AmpliTaq Gold polymerase, 1 x AT Gold buffer	94°C-6 min; 45 cycles (94°C-1 min, 55°C-1 min, 72°C-2 min); 72°C-10 min	1100 bp
Lr20	STS638	0.2 mM dNTP, 0.2 μM each Primer, 1.5 mM $MgCl_2$, 0.8 U Taq polymerase, 1 x Taq buffer,	94°C-3 min; 40 cycles (94°C-1 min, 62°C-1 min, 72°C-1 min); 72°C-10 min	540 bp
Lr21	KSUD14	0.2 mM dNTP, 0.5 μM each Primer, 2.125 mM $MgCl_2$, 0.8 U AmpliTaq Gold polymerase, 1 x AT Gold buffer	94°C-8 min; 35 cycles (94°C-30 s, 54°C-1 min, 72°C-1 min); 72°C-10 min	669 bp
Lr25	Lr25-F20/-R19	0.2 mM dNTP, 0.2 μM each Primer, 2.125 mM $MgCl_2$, 0.8 U AmpliTaq Gold polymerase, 1 x AT Gold buffer	94°C-15 min; 35 cycles (94°C-1 min, 55°C-1 min, 72°C-1 min); 72°C-10 min	1800 bp
Lr29	Lr29-F24/-R24	0.4 mM dNTP, 0.5 μM each Primer, 2.5 mM $MgCl_2$, 0.8 U AmpliTaq Gold polymerase, 1 x AT Gold buffer	94°C - 3 min; 35 cycles (94°C-1 min, 61°C-1 min, 72°C-2 min); 72°C-10 min	900 bp
Lr35	SR39-F/-R	0.2 mM dNTP, 0.9 μM forward primer, 1.15 μM reverse primer, 2.125 mM MgCl2, 1.0 U AmpliTaq Gold, 1 x AT Gold buffer	94°C – 5 min; 35 cycles (94°C-1 min, 60°C-1 min, 72°C-1 min); 72°C-10 min	900 bp
Lr37	Ventriup/LN2	0.4 mM dNTP, 0.5 μM each Primer, 2.5 mM $MgCl_2$, 0.8 U AmpliTaq Gold polymerase, 1 x AT Gold buffer	94°C–10 min; 35 cycles (94°C-1 min, 64°C-1 min, 72°C-1 min); 72°C-10 min	259 bp
Lr52	TXW200	0.2 mM dNTP, 0.2 μM each Primer, 1.5 mM $MgCl_2$, 0.8 U Taq polymerase, 1 x Taq buffer,	1 cycle (94°C–1 min, 58°C–1 min, 72°C–1 min); 30 cycles (94°C–30 s, 58°C–30 s, 72°C–30 s); 72°C-5 min	200 bp

amplified the specific fragments only in those 'Thatcher' NILs or selected wheat accessions carrying the corresponding *Lr* gene, i.e. they turned out to be specific for the respective gene. In contrast to this, the fragment of 570 bp specific for *Lr28* was also amplified in additional lines not carrying *Lr28* and was therefore excluded from further analysis (Table 2).

Verification of DNA markers in wheat genotypes

For validating PCR markers, these were further studied on wheat genotypes of diverse genetic backgrounds with postulated leaf rust resistance genes. The genotypes tested for *Lr1*, *Lr20*, *Lr21/Lr40*, *Lr25*, *Lr26*, *Lr34*, *Lr35*, *Lr37*, *Lr39/41*, *Lr47*, *Lr51* and *Lr52* showed marker alleles indicative of the predicted corresponding leaf rust resistance gene (Table 2). However, the amplification product of the *Lr52* marker (200 bp) was not only detected in the accession carrying this gene but also in other wheat accessions that were supposed to lack this gene.

Lr25 was detected in one (PI 636140) out of three accessions carrying *Lr25* as inferred from pedigree data. *Lr26* was postulated to be present in seven accessions. The specific STS marker fragment was identified in six of these genotypes, with the exception of cv. 'Apollo'. For the *Lr51* marker validation only one accession (F.7.3) was available. Among the 36 tested accessions, the specific CAPS marker fragment was detected only in this *Lr51* carrying line (Table 2).

Screening of DNA markers for Lr genes in German winter wheat cultivars

Based on these results the molecular markers, with the exception of markers for *Lr28* and *Lr52* which turned out to be non-specific, were screened on 115 German winter wheat cultivars for the presence or absence of the corresponding *Lr* genes.

Pedigree data of the investigated wheat cultivars suggested that the leaf rust resistance genes *Lr9*, *Lr19*, *Lr20*, *Lr21*, *Lr24*, *Lr25*, *Lr29*, *Lr35*, *Lr39*, *Lr47* and *Lr51* were not present in these cultivars (Table 1). Marker studies confirmed the absence of these genes. The markers did not amplify the specific marker fragments in any of the wheat cultivars (Table 1).

According to pedigree data, it was expected that *Lr1, Lr3* and *Lr34* would be present only in some of the cultivars, while *Lr10*, *Lr13*, *Lr14*, *Lr17, Lr26* and *Lr37* would occur frequently. Markers for *Lr1*, *Lr10*, *Lr26*, *Lr34* and *Lr37* were analysed in the set of winter wheat, while due to the lack of closely-linked PCR based markers, *Lr3*, *Lr13*, *Lr14* and *Lr17* could not be evaluated. The *Lr1* specific SNP was detected in four varieties: in cv. 'Travix' in accordance with pedigree data and in three non-postulated cvs ('Cortez', 'Madrid', 'Limes'). The presence of *Lr1* in 'Excellenz', 'Elegant' and 'Carenius' as predicted from pedigree could not be confirmed by marker analysis.

The specific marker fragment for *Lr10* was identified in 35 varieties, whereas pedigree data postulated *Lr10* in 46 accessions. Among these 46 varieties *Lr10* was detected in 27 varieties using the molecular marker. On the other hand, the *Lr10* marker fragment was present in 6 cultivars in which *Lr10* was not predicted from pedigree data and in 2 cultivars where the pedigree was unknown.

According to pedigree data, *Lr26* was postulated in 43 cultivars. The *Lr26* marker verified the presence of this gene in 14 cultivars. Furthermore, marker analysis identified *Lr26* in the cultivar 'Mirage' for which no pedigree data are available and in 'Sobi' and 'Champion' not possessing *Lr26* according to pedigree data.

The presence of *Lr34* was postulated in two cultivars, namely 'Ramiro' and 'Wenga' which could be confirmed by marker analysis. Using marker analysis, 37 out of 40 cultivars in which *Lr37* was postulated according to pedigree data showed the specific *Lr37* fragment. Additionally, the *Lr37* marker identified the gene in 9 cultivars where the presence of *Lr37* was not expected from pedigree data and in 2 cultivars where the pedigree data were unknown. In total *Lr37* was detected in 48 out of 115 analysed wheat cultivars.

Leaf rust monitoring inf field trials

To evaluate the reliability of pedigree data, cultivars for which information on *Lr26* in pedigrees differed from marker analysis were rated for leaf rust symptoms. The results from years 2004 to 2008 are displayed in Fig. 1 and show ratings for cultivars carrying *Lr26* ('Petrus' and 'Vergas') suggested by marker analysis and a cultivar carrying *Lr26* ('Empire') only suggested by pedigree. All cultivars carrying *Lr26* confirmed by marker analysis show lower infection rates than the cultivar without or the one carrying *Lr26* predicted by pedigree data, only (Fig. 1).

Leaf rust ratings of cultivars 'Thatcher' and 'Borenos' were used for calculations of percent infection of NILs and cultivars carrying respective *Lr* loci. Phenotypic evaluation after the scheme of James (1971) showed infection scores from 35.0± 10.0 (in 2004) to 75.0±10.0 (in 2007) on leaves of cultivar 'Borenos' and from 50.0±0.0 in 2004 to 65.0±10 in 2007 on leaves of 'Thatcher', without significant differences between these cultivars (Fig. 2). Incidence of leaf rust at Aschersleben and Quedlinburg was similar, with the infection scores of

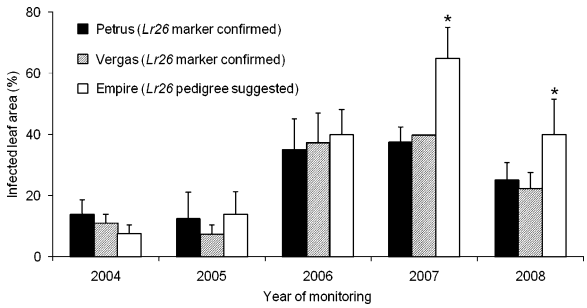

Fig. 1 Comparison of leaf rust infection scoring of cultivars carrying *Lr26* suggested by pedigrees and confirmed by markers. For the respective year of monitoring, one way ANOVA was performed to compare susceptibility between Petrus, Vergas (*Lr26* marker confirmed) and Empire (*Lr26* suggested by pedigree), Asterisks (*) mark significant differences between cultivars ($p<0.05$)

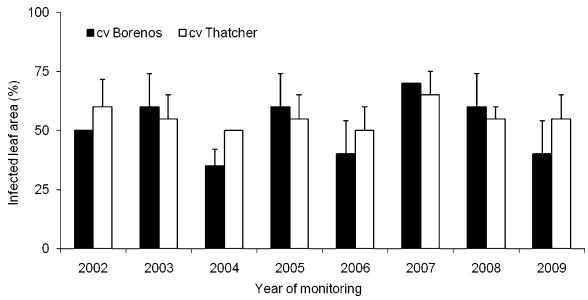

Fig. 2 Leaf rust infection scoring (James 1971) of cv. 'Borenos' and cv. 'Thatcher' in different years. One way ANOVA was performed to compare infection scores of these cultivars for every year of monitoring. Significant differences between infections of cultivars at a p level <0.05 are shown by one asterisk above the respective bars (*)

cv. 'Borenos' and 'Thatcher' (standards) 56.3±6.3 and 52.5±15.0, respectively at Aschersleben (2002–2005), and 55.0±4.1 and 51.3±11.8 respectively at Quedlinburg (2006–2009). Ratings of 10 *Lr* loci present in German cultivars showed significant changes of rust infections in 'Thatcher' NILs in field trials in the period 2002–2009 (Table 5).

The infection levels of NILs possessing a certain *Lr* gene related to the reference cultivar 'Thatcher' (100%±0.0) were significantly lower for *Lr13* (60.0%±0.0), *Lr14* (60.0%±0.0), *Lr17* (30.0%±0.0), *Lr34* (60.0%±0.0) and *Lr37* (15.0%±7.1) in 2002 whereas *Lr1*, *Lr3*, *Lr10*, *Lr16* and *Lr26* carrying NILS were on the same level as 'Thatcher'. Surprisingly, none of these *Lr* genes led to a qualitative resistance without symptoms in any year of observations. An abrupt change of infection scores was visible between 2002 and 2009 for all investigated *Lr* genes except *Lr34* in relation to cv. 'Thatcher' and also significant differences between years were observed (indicated by asterisks in Table 5). *Lr13* remained at a lower infection level in relation to 'Thatcher' from 2002 to 2006 ($p<0.041$) except in 2005. In 2007, 2008 and 2009 NILs that carry *Lr13* turned out to be as susceptible as 'Thatcher' (2007 and 2008, $p=0.694$; 2009 $p=1.000$). In these years also, changes in the infection level of *Lr14* and *Lr37* were observed. During only one growing season did the NIL *Lr14* show a switch of rating from 37.5%± 17.7 in 2006 ($p<0.001$) to 100.0%±0.0 in 2007 ($p=$ 1.000), and *Lr37* from 18.8±8.8 ($p<0.001$) to 85.7± 20.2 ($p=0.158$), in comparison to cultivar 'Thatcher'. These observations were confirmed by the direct comparison of infection scores between years 2006 and 2007 which were significantly higher in 2007 (see asterisks behind p-values) for all listed *Lr* genes except *Lr1* ($p=0.174$ between 2006 and 2007) and *Lr34* ($p=0.482$ between 2006 and 2007). Since 2007 no significant differences between the *Lr37* carrying NIL and 'Thatcher' have been detected ($p>0.158$). In addition, other monitored *Lr* loci such as *Lr26* displayed extreme alterations of susceptibility, indicated in 2006 by a rating of 12.5%±0.0 ($p<0.001$) and in 2007 by 85.7%±20.2 ($p=0.964$). Moreover, all observed NILs carrying *Lr* loci except *Lr34* shift to a higher susceptibility related to cultivar 'Thatcher' in 2007. Only NILs that contain *Lr17* and *Lr34* displayed lower symptom rates, of leaf rust in all years of investigation. The highest infection rate in the NIL carrying *Lr17* related to cv. 'Thatcher' was observed in 2008 with 58.3%±11.8 ($p<0.001$). In all investigated years *Lr34* confers also quantitative resistance, with a rating from maximal 67.6%±0.0 ($p=0.035$) in 2003 to a minimal 16.7%±7.9 in 2004 ($p<0.001$) in relation to cv. 'Thatcher'. In summary the comparison of infection rates between years showed alterations in susceptibility of all *Lr* genes but particularly from 2006 to 2007. Only two of ten analyzed *Lr* loci confer red significantly lower susceptibility in all years of monitoring and no NILs without any symptoms of leaf rust were detected. *Lr3* and *Lr10* were ineffective in 5 and 6 years, and *Lr16* in 7 out of 8 years of investigation. In 2007 a significant switch of *Lr14* and *Lr37,* which had conferred a certain level of resistance till 2006, to higher susceptibility was observed.

The investigated cultivars carry mostly combinations of *Lr* genes with the exception of some that carry only one *Lr* gene. Phenotypic evaluation of leaf rust resistance on 'Cortez' (*Lr1*), 'Semper' (*Lr3*), 'Monopol' (*Lr10*), 'Akratos', 'Lucius', 'Ritmo', 'SW Topper' (*Lr13*), 'Alidos' (*Lr14*), 'Petrus', 'Vergas' (*Lr26*) and 'Amply', 'Manager', 'Noah', 'Tommi', 'Tuareg', 'Türkis' (*Lr37*) show, similarly to the NILs carrying corresponding *Lr* genes, a clear trend to higher susceptibility from 2006 to 2007 relative to the reference cultivar 'Borenos' (Table 1). Ratings displayed higher infection levels for cv. 'Cortez' from 2006 to 2007 ($p=0.097$) and 'Semper' ($p<0.001$). The cv. 'Monopol', in which *Lr10* is not confirmed by pedigree, shows high infection rates without significant differences ($p=0.228$). *Lr13* carrying cultivars showed higher infection rates in 2007, as with

Table 5 Scorings of NILs carrying the same *Lr* genes as the investigated cultivars. All scorings of Thatcher are averaged as 100%, data of NILs carrying respective *Lr* loci show percentage of leaf rust infection relativ to cultivar Thatcher. Significant differences of percentage infection related to cv.Thatcher with p-values <0.05 are printed in bold. Asterisks indicate significant changes of infection scores in comparison to the year before

Year	2002		2003		2004		2005		2006		2007		2008		2009	
NIL	Infection rate (%)	p-value	Infection rate (%)	p-value	Infection rate (%)	p-value	Infection rate (%)	p-value	Infection rate (%)	p-value	Infection rate (%)	p-value	Infection rate (%)	p-value	Infection rate (%)	p-value
Thatcher	100.0%±0.0		100.0%±17.0		100.0%±0.0		100.0%±17.0		100.0%±25.0		100.0%±0.0		100.0%±17.0		100.0%±25.0	
Lr1	80.0%±28.3	0.795	**50.0%±0.0**	**<0.001**	77.8%±15.7	0.478	50.0%±0.0	0.108	**25.0% ±17.7**	<0.001	71.4%±0.0	0.158	**50.0%±0.0**	**0.001**	**43.8%±8.8**	**0.004**
Lr3	100.0%±0.0	1.000	**41.7%±11.8**	**<0.001***	77.8%±15.7	0.478	100.0%±23.6	1.000	**25.0% ±0**	<0.001	100.0%±0.0	1.000*	83.0%±0.0	0.144	75.0%±0.0	0.378
Lr10	120.0%±28.3	0.795	**66.7%±0.0**	**0.014**	**55.6%±15.7**	**<0.004**	100.0%±23.6	1.000	**37.5%±17.7**	<0.001	100.0%±0.0	1.000*	116.7%±0.0	0.694	75.0%±0.0	0.715
Lr13	**60.0%±0.0**	**0.041**	**29.2%±5.9**	**<0.001***	**27.8%±7.9**	**<0.001**	50.0%±0.0	0.108	**18.8%±8.8**	<0.001	85.7%±20.2	0.964*	116.7%±0.0	0.694	87.5%±17.7	1.000
Lr14	**60.0%±0.0**	**0.041**	**66.7%±0.0**	**0.014**	**38.9%±7.9**	**<0.001**	**37.5%±17.7**	**0.019**	**37.5%±17.7**	<0.001	100.0%±0.0	1.000*	**66.0%±23.6**	**0.014**	75.0%±0.0	0.715
Lr16	100.0%±0.0	1.000	83.3%±0.0	0.644	77.8%±15.7	0.478	83.3%±47.1	0.999	**50.0% ±0.0**	0.010	100.0%±0.0	1.000*	75.0%±11.8	0.144	100.0%±35.4	1.000
Lr17	**30.0%±0.0**	**<0.001**	**8.3%±0.0**	**<0.001***	**6.7%±0.0**	**<0.001**	**25.0%±0.0**	**0.003**	**10.0% ±3.5**	<0.001	**46.4%±15.2**	**<0.001***	**58.3%±11.8**	**<0.001**	**18.8%±8.8**	**<0.001**
Lr26	100.0%±0.0	1.000	**66.7%±0.0**	**0.014**	**55.6%±15.7**	**0.004**	66.7%±23.6	0.597	**12.5% ±0.0**	<0.001	85.7%±20.2	0.964*	75.0%±11.80	0.144	68.8%±8.8	0.224
Lr34	**60.0%±0.0**	**0.041**	**67.6%±0.0**	**0.035**	**16.7%±7.9**	**<0.001***	**33.3%±0.0**	**0.010**	**37.5%±17.7**	<0.001	**64.3%±10.1**	**0.003**	**60.5%±6.7**	**0.026**	**53.3%±5.8**	**0.026**
Lr37	**15.0%±7.1**	**<0.001**	**20.8%±5.9**	**0.002**	**6.7%±0.0**	**<0.001**	**25.0%±11.8**	**0.003**	**18.8%±8.8**	<0.001	85.7%±20.2	0.158*	66.7%±0.0	0.144	68.8%±8.8	0.378

'Akratos' (p=0.007), 'Lucius' (p=0.003), 'Ritmo' (p=0.040), 'SW Topper' (p=0.003). 'Alidos' which carries *Lr14* showed significant changes (p<0.001). The infection rate of *Lr26* carrying cv. 'Petrus' increased from 2006 to 2007 (p=0.018), but not that of 'Vergas' (p=0.104). Simultaneously, all cultivars carrying only *Lr37* shifted to higher infection rates from 2006 to 2007, as with 'Amply' (p=0.006), 'Manager' (p=0.018), 'Türkis' (p=0.015), 'Tommi' (p<0.001), 'Noah' (p=0.032) except 'Tuareg' (p=0.066). Resistance based on *Lr1* which conferred significantly lower susceptibility in NILs trials in 2006 was detected in cultivars 'Madrid' (combined with *Lr13, Lr14*), 'Travix' (combined with *Lr10*, *Lr26*, *Lr37*), and 'Limes' (combined with *Lr10*, *Lr13*, *Lr26*). These cultivars remained at low infection levels as no significant changes were observed from 2006 to 2007 in cv. 'Madrid' (p=0.133), cv. 'Travix' (p=0.065) and 'Limes' (p=0.384). As in the monitoring of single *Lr* loci in NILs, combinations of ineffective resistances resulted in partial susceptibility to leaf rust. Thus cultivars combining *Lr10* and *Lr13* showed a partial switch to higher infection scores from 2006 to 2007, as with 'Dekan' (p=0.003), 'Greif' (p=0.008) and 'Motiv' (p=0.334) which showed a high infection score already in 2006. If effective resistance genes such as *Lr17* were combined with ineffective *Lr* loci *Lr3*, *Lr10, Lr13* or *Lr26*, as detected in cultivars like 'Frodin', 'Gaston', 'Meteor', 'Milvus', no significant differences between the ratings in 2006 and 2007 (p-values between 0.058 and 0.874) were observed. An exception was cv. 'Brilliant', showing increased infection rates at a low level but significant (p=0.024). Combination of leaf rust resistances with *Lr34* resulted, similarly to the NILs carrying *Lr34, in* a stable lower infection rate as demonstrated by the cultivar 'Wenga' which carried *Lr14*, *Lr26*, *Lr34* and no changes of scorings were detected between 2006 and 2007 (p=0.843, Table 1). Cultivars carrying *Lr34* alone are not grown in Germany. The combination of at least 4 *Lr* genes as in cvs 'Brilliant' (see above), Elegant (p=0.143) and 'Travix', in which *Lr1*, *Lr10*, *Lr26* and *Lr37* are confirmed by markers, is an effective strategy to achieve low infection levels. However increasing infection scores between 2006 and 2007 were also observed, although at a lower level.

Ratings of NILs and cultivars indicate clearly the necessity to combine *Lr* loci in cultivars to maintain

resistance against wheat leaf rust. Investigations show that the application of specific molecular markers is essential for confirmation of *Lr* genes incorporated into wheat cultivars due to the possibility of false pedigree data.

Discussion

Ten out of twelve molecular markers closely linked to *Lr* genes showed a high specificity in NILs and different genetic backgrounds. Due to false positive reactions in verification tests the *Lr52* marker is not suited for a diagnostic identification of this gene. The marker for *Lr25* did not detect this gene in the accessions Citr 14189 and Citr 17927 as would be predicted from pedigree. However, we could not find any information on whether this gene was really transferred to these accessions. For the cultivar 'Apollo', supposed to carry *Lr26,* we could not identify the specific amplification product for this gene. The same result was reported by Stępień et al. (2003). In summary, molecular markers for different *Lr* genes verified in this (*Lr1*, *Lr20*, *Lr21*, *Lr25*, *Lr26*, *Lr34*, *Lr35*, *Lr37*, *Lr39*, *Lr47*, *Lr51*) and previous (*Lr9*, *Lr10*, *Lr19*, *Lr24*, *Lr29*) studies (references in Table 3) are an effective tool to assess the presence of these genes in wheat and can be used in marker-assisted selection. This conclusion is confirmed by our result comparing infection scores, molecular marker analyses and pedigree data for the presence of *Lr26* (Fig. 1). In particular,, higher infection rates for cultivar 'Empire' in 2007, 2008 can only be explained by results from marker analyses that determine the lack of *Lr* genes in contrast to the pedigree information. Furthermore, only marker results indicating *Lr37* in cultivars 'Manager', 'Tommi', 'Tuareg' and 'Türkis' are consistent with the lower infection rates for this cultivars compared to the cultivar 'Dream' which does not carry *Lr37* as inferred from pedigree and marker analyses (Table 1).

Many authors conclude there is a greater predictive ability of molecular markers than pedigree data (Błaszczyk et al. 2008; Stępień et al. 2003). Our results clearly indicate the advantage of molecular markers for evaluating the presence of *Lr* genes in wheat cultivars compared to pedigree data and are in accordance with numerous studies and reviews (Stępień et al. 2003; Ordon et al. 2004).

The genetic variability of the leaf rust resistance in German wheat cultivars is low; only seven out of twenty investigated *Lr* genes are present in current winter wheat cultivars. Only a few resistance genes such as *Lr1*, *Lr3*, *Lr10*, *Lr13*, *Lr14*, *Lr17*, *Lr26* and *Lr37* are individually or in combination widely used in 93 out of 115 cultivars. The *Lr1* resistance, which has been applied for many years world wide was detected by SNP markers in the cultivars Cortez, Travix, Madrid and Limes, partially confirmed by pedigrees. This gene conferred stable resistance in 2006 and 2007 in combination with *Lr10*, *Lr13*, *Lr26* and *Lr37*. Cultivars possessing *Lr1* show no significant changes in the level of resistance from $p=0.065$ (cv.' Travix') to $p=0.384$ (cv. 'Limes') between years 2006 and 2007 (Table 1). However, *Lr1* and *Lr3* are race specific genes causing hypersensitive spots and pathotypes which are virulent to other resistance genes mostly comprise virulence to *Lr1* (Cloutier et al. 2007). The selection of isolates virulent to *Lr1* depends on the growing area of cultivars possessing *Lr1* and is not associated with spontaneous mutations within leaf rust populations (Kolmer 1992). *Lr3* is not used alone but only in combination with at least two other *Lr* genes, simply because *Lr3* alone does not mediate resistance. According to the results of screening NILs carrying *Lr3*, Manisterski et al. (2000) detected a frequency of virulence in 250 *Puccinia triticina* isolates between 35.0% and 78.3% on the cultivar 'Democrat' which carries only *Lr3*. These data were confirmed by Goyeau et al. (2006), which detected a frequency of virulence between 44.0% and 66.0% in France. *Lr10* and *Lr13* present in 9 German cultivars are effective only in combination with additional *Lr* -loci and are not effective in Australia and South America (Pathan and Park 2006). Using the specific STS marker, *Lr10* was detected in our investigations in 35 and *Lr13* was defined by pedigree data in 72 cultivars. The high proportion of cultivars carrying these *Lr* loci is accompanied by the selection of virulent pathotypes of leaf rust. Accordingly, Park et al. (2001) sampled isolates across Europe and detected a frequency of virulence to *Lr10* ranging from 4.6% to 94.3%. In agreement with these results we could find significant differences of NILs that carry *Lr10* in comparison to the susceptible cultivar 'Thatcher' only in 2003, 2004 and 2006. Evidence for the adaptation of rust populations after the release of new cultivars is

provided by cv. 'Mannitou' which carries *Lr13*; this cultivar was completely resistant in Canada in 1966 but turned out to be partially susceptible in 1987 (Kolmer 1992). On the other hand, *Lr13* enhances the effectivity of other resistance genes in combination, such as *Lr34* (Kolmer 1992). The excessive use of only one resistance gene like *Lr10*, *Lr13* and *Lr37* provokes selection for leaf rust pathotypes which overcome the respective resistance genes. Pathotypes of *Puccinia triticina* present in fields possess virulence to more than one *Lr* locus, so that many resistance loci are affected by such isolates. This is confirmed by Park et al. (2001) for pathotype 122–1,3,4,6,7 which has virulence to *Lr1, Lr2a, Lr2c, Lr3, Lr10, Lr11, Lr14a, Lr15, Lr17, Lr20, Lr27, Lr31*, and by Goyeau et al. (2006) for *Lr2*, *Lr3*, *Lr10*, *Lr13*, *Lr14*, *Lr15*, *Lr17*, *Lr26*, and *Lr37*. According to the acreage sown to a specific variety, virulent races were selected with respect to *Lr13* (Goyeau et al. 2006) in France and Europe (Park et al. 2001). Hanzalová et al. (2008) obtained similar results for *Lr26* which was overcome in the early seventies by race SaBa77. Nevertheless *Lr26* is present in 18 (marker confirmed) current cultivars. Our ratings confirm the presence of virulent races in all years of monitoring, e.g. the *Lr26* carrying NIL turned out to be significantly less susceptible than 'Thatcher' only from 2004 to 2006. In our investigations *Lr37* was detected in 48 out of 115 varieties. Of these, cv. 'Tommi' and 'Punch' carrying *Lr37* were grown on a large acreage and turned out to be resistant only untill 2006. *Lr37* was derived from the wild relative of wheat *Aegilops ventricosa* (Goyeau and Lannou 2010) and was transferred to the French line 'VPM1' which has beed widely used in wheat breeding. Virulence to *Lr37* was reported for the first time in Western Australia in 2002 (Pathan and Park 2006). Most of the leaf rust resistance genes present in European cultivars are race specific (Goyeau and Lannou 2010) and have been overcome, as with *Lr10*, *Lr13* and *Lr37*. The resistance gene *Lr14* has been introduced in 36 cultivars in combination and in two cultivars, i.e. 'Alidos' and 'SW Maxi' individually. The 'Thatcher' NIL carrying this resistance gene expressed quantitative resistance in all years of monitoring except 2007 and 2009. However, there are significant fluctuations of the infection rates of the *Lr14*-NIL (Table 5) which indicate a variable portion of Lr14-virulent races in the leaf rust population. Such sudden changes of virulence within one year suggest a race specific character of the resistance, contrary to the assumption of Schnurbusch et al. (2004), that quantitiative resistance conferred by this gene may be due to defence mechanisms at the molecular level similar to *Lr34*. Differences of monitoring data of NILs between *Lr14* and *Lr 34* in 2007 (Table 5) are in accordance with Herrera-Foessel (2008) who described *Lr14* as race specific, and Krattinger et al. (2009) who demonstrated that *Lr34* is nonspecific and durable as it confers slow rusting resistance. The level of *Lr34* mediated resistance for leaf rust infection correlates with the development of leaf tip necrosis and is associated with reduced intercellular hyphal growth leading to reduced uredinium sizes but not with a hypersensitive response or papilla formation (Krattinger et al. 2009). *Lr34* was detected in our tests in 'Chinese Spring' and in the cultivars 'Ramiro' and 'Wenga'. The detection by PCR marker cssfr5 (Lagudah et al. 2009) corresponds to pedigree data of cvs. 'Ramiro' and 'Wenga'. Lagudah et al. (2009) and Krattinger et al. (2009) also detected *Lr34* by marker analysis in 'Chinese Spring'. Varieties and the NIL containing *Lr34* revealed partial resistance from 2000–2009 and confirm previous investigations which characterized *Lr34* as durable (Krattinger et al. 2009). However, *Lr34* is the only resistance characterized as race nonspecific in German cultivars. Another resistance gene described as non race specific is *Lr46* which up to now has not been used in German cultivars, but has conferred resistance to leaf and stripe rust for 30 years and was detected in cultivar 'Pavon 76' by Singh et al. (1998).

An example of a stable race specific resistance is *Lr17* present in only 8 German cultivars, exclusively together with *Lr13*. In the NIL trials (Table 5) *Lr17* confers resistance in all years of monitoring. The NIL carrying *Lr17* showed a lower infection rate than the one carrying *Lr34* suggesting a high durability of this resistance. However, Kolmer (2005) collected *Lr17* virulent isolates in the prairie region of Manitoba, independent of the presence of cultivars carrying *Lr17* in this region. Many studies have concluded that races with specific virulence to *Lr* loci exist on susceptible cultivars in low frequency dominated by the most aggressive pathotype (Kolmer 2005) and are selected by the growing acreage of race specific *Lr* carrying cultivars (Hanzalová et al. 2008;, Park et al. 2001). Hence, it may be expected that rust populations

containing races virulent against *Lr17* will be more frequent if the acreage of *Lr17* carrying cultivars increases in Germany. Our investigations showed sudden changes of susceptibility in cultivars that carry single vertical resistances. Leaf rust resistance genes from *Lr1* to *Lr60* (Bolton et al. 2008) are described and some of these confer complete resistance which up to now is not overcome in Germany, e.g. *Lr9* and *Lr19* in our studies (data not shown, Hanzalová et al. 2008). However, it is expected that these resistances will not be durable when widely applied in cultivars, e.g. a pathotype virulent on *Lr9* has been described already by Kolmer (2005).

Our results displayed in Table 1 suggest, that the combination of vertical resistances in cultivars is also an effective strategy to avoid the breakdown of leaf rust resistance genes. However, Park et al. (2001) and Goyeau et al. (2006) concluded that in addition to the resistance genes employed in cultivars and the growing area, factors including aggressiveness, tolerance to fungicide treatment and climatic conditions were responsible for the dominance of specific pathotypes.

Nevertheless, for combining resistance genes to obtain more durable and longer lasting resistances, molecular markers closely linked to *Lr* genes are an effective, reliable and diagnostic tool as shown in this study. Additional new races were selected in a gene-for-gene manner by growing wheat cultivars carrying only a few resistance genes over extensive acreages (Goyeau et al. 2006). The low number of *Lr* loci in German cultivars which confer race specific resistance, with the exception of *Lr34*, has led to the selection of virulent races. This is demonstrated by the breakdown of resistance genes such as *Lr26* and *Lr37* after a few years of cultivating varieties carrying these genes over a large acreage. Therefore, new sources of durable non race specific leaf rust resistance have to be employed in wheat breeding. They have been identified e.g. in *Aegilops sharonensis*, *Aegilops geniculata* and additional wild relatives including *Aegilops tauschii, Triticum monococcum, Triticum boeoticum*, *Triticum dicoccum and Triticum turgidum*, *Aegilops longissima* and cultivated *Triticum durum* (Valkoun 2001). However, the transfer of such leaf rust resistance genes in wheat, particularly from diploid progenitors like *Triticum monococcum* and *Triticum boeticum* is a protracted procedure.

Acknowledgements We thank Dr. D. Rentel for providing cultivars and N. Pastor-Käppner, P. Remus, J. Mangelsdorf, I. Renneberg and M. Weilepp for analysis of *Lr* loci and rating of leaf rust symptoms in field trials.

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