

The durable wheat disease resistance gene Lr34 confers common rust and northern corn leaf blight resistance in maize

Peter Rogowsky, Justine Sucher, Rainer Boni, Ping Yang, Heike Buchner, Christine Kastne, Jochen Kumlehn, Simon Krattinger, Beat Keller

▶ To cite this version:

Peter Rogowsky, Justine Sucher, Rainer Boni, Ping Yang, Heike Buchner, et al.. The durable wheat disease resistance gene Lr34 confers common rust and northern corn leaf blight resistance in maize. Plant Biotechnology Journal, 2017, 15 (4), pp.489-496. 10.1111/pbi.12647 . hal-01602570

HAL Id: hal-01602570 https://hal.science/hal-01602570

Submitted on 25 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - ShareAlike 4.0 International License

The durable wheat disease resistance gene *Lr34* confers common rust and northern corn leaf blight resistance in maize

Justine Sucher¹, Rainer Boni¹, Ping Yang¹, Peter Rogowsky², Heike Büchner³, Christine Kastner³, Jochen Kumlehn³, Simon G. Krattinger^{1,*} and Beat Keller^{1,*}

¹Institute of Plant and Microbial Biology, University of Zurich, Zurich, Switzerland ²ENS, UMR 5667- RDP, Lyon, France

³Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

Received 9 August 2016; revised 3 October 2016; accepted 11 October 2016. *Correspondence (Tel +41 44 634 82 30; fax +41 44 634 82 04; email bkeller@botinst.uzh.ch (BK)) and (Tel +41 44 634 82 33; fax +41 44 634 82 04; email skratt@botinst.uzh.ch (SGK))

Keywords: durable disease resistance, fungal pathogens, *Lr34/Yr18/Sr57/ Pm38*, Maize, common rust, northern corn leaf blight.

Summary

Maize (corn) is one of the most widely grown cereal crops globally. Fungal diseases of maize cause significant economic damage by reducing maize yields and by increasing input costs for disease management. The most sustainable control of maize diseases is through the release and planting of maize cultivars with durable disease resistance. The wheat gene Lr34 provides durable and partial field resistance against multiple fungal diseases of wheat, including three wheat rust pathogens and wheat powdery mildew. Because of its unique qualities, Lr34 became a cornerstone in many wheat disease resistance programmes. The Lr34 resistance is encoded by a rare variant of an ATP-binding cassette (ABC) transporter that evolved after wheat domestication. An Lr34-like disease resistance phenotype has not been reported in other cereal species, including maize. Here, we transformed the Lr34 resistance gene into the maize hybrid Hi-II. Lr34-expressing maize plants showed increased resistance against the biotrophic fungal disease common rust and the hemi-biotrophic disease northern corn leaf blight. Furthermore, the Lr34-expressing maize plants developed a late leaf tip necrosis phenotype, without negative impact on plant growth. With this and previous reports, it could be shown that Lr34 is effective against various biotrophic and hemi-biotrophic diseases that collectively parasitize all major cereal crop species.

Introduction

Maize (corn) is one of the most widely grown crops, and maize production passed the mark of 1000 million metric tons in 2013 (FAO, 2016; de Lange *et al.*, 2014). Maize is used for human consumption, as a major source of animal feed (Chaudhary *et al.*, 2014) and for the production of a number of industrial products such as starch, oil, ethanol and malt. Maize is grown in more than 160 countries over a wide range of agro-climatic zones.

Fungal diseases are a major threat to maize production, resulting in reduced yields and high input costs for disease control. Global maize yield losses caused by diseases were estimated at about 9% on average in 2001–2003 (Oerke, 2006). Sixty-two maize-infecting diseases have been described, and among them, sixteen have been identified as major constraints for maize production (Chaudhary et al., 2014). The hemi-biotrophic fungal disease northern corn leaf blight (NCLB), also known as Turcicum leaf blight (Exserohilum turcicum), and the maize common rust disease caused by the biotrophic fungus Puccinia sorghi are among these most important foliar diseases (Munkvold and White, 2016). Biotrophic and hemi-biotrophic fungi have different lifestyles. Obligate biotrophs such as rusts and powdery mildews exclusively grow and reproduce on living plant tissue (Bettgenhaeuser et al., 2014; Eckardt, 2006). In contrast, hemi-biotrophic fungi feed on dead leaf tissue after an initial biotrophic phase that can last from a few hours to several days.

The release of cereal cultivars with high levels of durable field resistance is one of the most effective and sustainable strategies to

control fungal diseases (McDonald and Linde, 2002). Hundreds of fungal disease resistance genes have been described in various cereal species (Singla and Krattinger, 2016; Wisser et al., 2006). Fungal disease resistance genes can be broadly classified based on their specificity and durability. Race-specific resistance (R) genes are only effective against some isolates of a pathogen, whereas race nonspecific resistance is effective against all pathogen isolates. The former is often controlled by a single major R gene encoding for plasma membrane-localized or intracellular immune receptors, and the latter is often the result of the additive action of several quantitative resistance genes with minor phenotypic effects (Krattinger and Keller, 2016). The deployment of single R genes in one cultivar often results in breakdown of disease resistance because of rapid pathogen adaptation. The combination of several race-specific R genes in one cultivar, the use of multiline cultivars with different resistance gene combinations and the use of more durable resistance sources are effective strategies to increase the durability of disease resistance in the field (Brunner et al., 2012).

In hexaploid bread wheat (*Triticum asestivum*), three genes have been described that confer race nonspecific resistance against multiple fungal diseases. These three genes, named *Lr34* (=Yr18/Sr57/Pm38), *Lr67* (=Yr46/Sr55/Pm46) and *Lr46* (=Yr29/Sr58/Pm39), confer partial resistance against all races of the fungal diseases caused by wheat leaf rust (*Puccinia triticin*), stripe rust (*P. striiformis* f.sp. *tritici*), stem rust (*P. graminis* f.sp. *tritici*) and powdery mildew (*Blumeria graminis* f.sp. *tritici*) (Ellis *et al.*, 2014; Spielmeyer *et al.*, 2013). In addition, these genes are associated with a senescence-like phenotype referred to as leaf

tip necrosis (LTN) (Krattinger *et al.*, 2016; Risk *et al.*, 2012). In wheat, LTN develops in the flag leaves at the heading stage under some environmental conditions (Singh and Huerta-Espino, 1997). Two of these genes, *Lr67* and *Lr34*, have been cloned and, respectively, encode a hexose transporter (Moore *et al.*, 2015) and a putative ATP-binding cassette (ABC) transporter (Dakouri *et al.*, 2010; Krattinger *et al.*, 2009). ABC transporters utilize ATP hydrolysis to translocate substrates across cellular membranes (Jasinski *et al.*, 2003; Rea, 2007).

Multiple allelic variants have been found for Lr34 and Lr67, but only one resistance-conferring variant referred to as Lr34res and Lr67res has been found for each gene, respectively. Interestingly, both Lr34res and Lr67res are the result of rare mutation events that occurred after domestication in ancient wheat landraces (Kolmer et al., 2008; Krattinger et al., 2013; Moore et al., 2015). The LR34res protein version differs by only two amino acid polymorphisms from the LR34sus protein of which one is critical for disease resistance (Chauhan et al., 2015; Krattinger et al., 2009). Bread wheat was domesticated around 10 000 years ago, long after wheat shared its last common ancestor with other cereal species. An Lr34-like disease resistance has so far not been described in other globally important cereals like rice and maize. Lr34res was introduced into modern wheat in the 1900s by the Italian wheat breeder Nazareno Strampelli and has since been extensively used for crop protection (Kolmer et al., 2008). In particular, the International Maize and Wheat Improvement Center (CIMMYT) continues to use combinations of partial rust resistance genes with additive effects in its rust improvement programme (Singh et al., 1991, 2005).

Orthologous Lr34 genes are present in rice and sorghum, but not in maize and in barley (Krattinger et al., 2011, 2013). However, only the susceptible haplotype has been found among these Lr34 orthologs which is in agreement with the very recent emergence of the Lr34res allele after wheat domestication. Despite the absence of the resistant Lr34 haplotype in cereals other than wheat, Lr34res was functionally transferred into barley and rice, where it provides resistance against the adapted barley pathogens barley leaf rust (Puccinia hordei) and barley powdery mildew (Blumeria graminis f.sp. hordei) as well as the rice-specific fungus rice blast (Magnaporthe oryzae), respectively (Krattinger et al., 2016; Risk et al., 2013). In barley, the expression of Lr34res resulted in high levels of disease resistance at the seedling stage. This was, however, accompanied by a strong LTN phenotype which had a negative impact on plant growth and yield (Chauhan et al., 2015; Risk et al., 2013). In rice, one of the transgenic lines showed a late LTN phenotype resulting in a plant development similar to the nontransgenic sib line but increased levels of rice blast resistance (Krattinger et al., 2016).

In this study, we transformed the Hi-II hybrid maize line with *Lr34res* driven by its native promoter. *Lr34res*-expressing maize lines showed an increased resistance against two of the most important foliar maize diseases: common rust and northern corn leaf blight.

Results

Lr34res confers common rust and northern corn leaf blight resistance in maize

Thirteen fertile independent primary transgenic (T0) plants were generated in the maize hybrid Hi-II by Agrobacterium-mediated transformation. DNA blot analyses indicated that these 13 plants carry at least two insertions of the *Lr34res* transgene (one line had two copies, seven lines had three copies, and five lines had more than three copies).

Three independent transgenic events, called 161, 163 and 164, respectively, were selected for further characterization. Each of them carried three co-segregating copies of *Lr34res* (Figure S1b), and they were chosen based on the development of a LTN phenotype and on the number of available T1 kernels. *Lr34res* and sib T1 plants were advanced to the T2 generation and homozygous T2 plants for events 163 and 164, and segregating T2 plants for event 161 were selected for further analyses. For event 161, all plants were analysed by PCR for the presence or absence of *Lr34res*.

Pathogenicity analyses were undertaken on these three independent T2 families using the biotrophic fungus common rust and the hemi-biotrophic fungus NCLB. For common rust, all three lines showed markedly increased resistance compared to their sibs lacking Lr34res. The plants derived from events 163 and 164 did not develop macroscopic symptoms 12 days after infection (d.a.i.), whereas their sibs showed brownish pustules typical for common rust. A few and smaller pustules were visible on Lr34resexpressing plants of event 161 (Figure 1a, Figure S2). We guantified fungal biomass in infected plant tissue using fluorescently labelled wheat germ agglutinin (WGA-FITC) that specifically binds to the fungal cell wall component chitin (Ayliffe et al., 2013). Similar to the macroscopic observations, the Lr34resexpressing maize plants of all three independent events analysed in detail had significantly reduced chitin levels compared to their sibs (Figure 1b). Chitin levels in event 161 were higher (0.93 μ g/ mg fresh weight) compared to plants of events 163 (0.10 μ g) and 164 (0.089 μ g), which is in agreement with the weaker macroscopic resistance phenotype in the former. However, even 161derived transgenics had much reduced chitin levels compared with their sibs lacking Lr34res (2.67–3.41 μ g).

NCLB symptoms were evaluated macroscopically ten and 14 d.a.i. *Lr34res*-expressing maize plants showed a delay in symptom development with smaller lesions compared to the sibs. At ten d.a.i., most of the Lr34res plants did not show visible signs of infection, whereas most of the sib plants already showed severe symptoms (Figure 1c). After 14 days, symptoms also appeared on Lr34res plants, but they were less severe than on sib lines (Figure S3). Disease severity was also quantitatively assessed by scoring the percentage of plants showing symptoms from eight to 13 d.a.i. and the area under the disease progress curve (AUDPC) was calculated according to Hurni et al. (2015). This quantification again revealed a delay in NCLB development in plants of the three Lr34res-expressing T2 families (Figure 1d). Our infection experiments showed that Lr34res confers a reduction in P. sorghi and E. turcicum development. The partial resistance phenotype is reminiscent of Lr34res' partial resistance phenotype in wheat.

Lr34res expression in maize results in LTN but no apparent growth reduction

In barley and in rice, high *Lr34res* expression levels at the seedling stage were associated with a strong LTN phenotype resulting in a negative impact on plant development (Krattinger *et al.*, 2016; Risk *et al.*, 2013). Among four transgenic rice events, one showed low *Lr34res* seedling expression levels coupled with late LTN development and no obvious growth penalty (Krattinger *et al.*, 2016). All three transgenic maize events developed a LTN phenotype appearing after ~6 weeks (Figure 2a). At the seedling stage, when the expression level was measured and the infection tests were performed, no LTN was



Figure 1 *Lr34res* provides partial resistance against common rust and NCLB in maize. (a) Macroscopic evaluation of common rust symptoms on seedling leaves of *Lr34res* transgenic maize event 164 and corresponding sib plant 12 days after infection. (b) WGA-FITC chitin quantification of common rust, 12 d.a.i. * indicates significant differences between *Lr34res*-expressing plant and sibs (Mann–Whitney–Wilcoxon test W = 25, P < 0.05). Error bars represent standard errors of five biological replicates. (c) Macroscopic observation of NCLB symptoms ten d.a.i on *Lr34res* event 164 and its corresponding sib line. (d) Area under the disease progress curve (AUDPC) of the different *Lr34res* transgenic individuals and sibs (pool of sib derived from the different events) calculated between day 8 to day 13 after infection. Error bars represent standard errors from at least three independent experiments. * indicates a significant differences to the sibs (Mann–Whitney–Wilcoxon test, W = 18 or 24, P < 0.05).

visible. Hence, the *Lr34res*-based resistance was apparent before LTN emerged.

Seedling expression levels of the three independent maize events were assessed by RT-qPCR. Interestingly, plants derived from events 163 and 164 had 774-fold and 816-fold higher Lr34res expression levels compared to those of event 161 (Figure 2b). As shown above, despite the relatively low Lr34res expression levels in event 161, it showed a clear NCLB and common rust resistance phenotype. To determine a possible negative impact of Lr34res on plant development, we measured two parameters: plant height and aboveground fresh weight of Lr34res-expressing maize plants and respective sibs grown under glasshouse conditions (Figure 3). These parameters appear to be good indicators of potential negative impacts of Lr34res on plant development as they show an obvious reduction in Lr34resexpressing barley plants and the high-expressing rice lines (Krattinger et al., 2016; Risk et al., 2013). In Lr34res-expressing maize plants, however, no significant differences were observed for these parameters compared with the respective sib lines. Also, visually there was no apparent impact on the plant development when Lr34res was expressed. These results indicate that unlike barley and rice, even a relatively strong expression of Lr34res as found in events 163 and 164 does not impact plant growth under our growth conditions.

Orthologous Lr34 genes in maize and distant relatives

Based on a comparative analysis, Krattinger et al. (2011) showed that the most similar maize homologue of the LR34 protein, GRMZM2G014282, was only 58% identical to LR34res. In contrast, rice and sorghum have more similar LR34 orthologs with 86% and 72% amino acid identity, respectively. To test for the presence of Lr34 orthologs in the Zea lineage (maize) and its sister taxa, the Tripsacum lineage, a PCR probe was developed based on sequences that are conserved among Lr34 sequences of wheat, rice and sorghum. This PCR probe spanned the critical sequence polymorphisms that distinguish Lr34res from Lr34sus. PCR was performed on genomic DNA of bread wheat (cv. Chinese Spring), rice (cv. Nipponbare), maize (Hi-II) and ten different Tripsacum species (Table S1). Wheat, rice and Tripsacum showed PCR amplification of the expected fragment size, but no amplification product was produced from maize (Figure S4a). The amplification products of Tripsacum were sequenced and showed greater than 72% nucleotide identity compared to the Lr34 wheat sequence. The corresponding fragment of the rice and sorghum orthologs, OsABCG50 and Sb01 g016775, showed 90% and 77% nucleotide identity, respectively, whereas no significant blast of this segment was obtained against the maize homologue GRMZM2G014282. All the Tripsacum sequences



Figure 2 Expression of *Lr34res* results in a LTN phenotype in the three transgenic lines. (a) Representative example of the third leaf of six-week-old seedlings showing a characteristic LTN phenotype. (b) Normalized *Lr34res* expression in transgenic lines. Data are normalized to the reference gene folylpolyglutamate synthase (FPGS). *Lr34res* expression level of line 161 = 1. Letters indicate line with equivalent expression level (Mann–Whitney–Wilcoxon *test, P* < 0.05). Errors bars represent standard errors from three biological replicates.

clustered within the '*Lr34* clade' in a phylogenic tree (Figure S4b), and all the *Tripsacum* species had the susceptible haplotype for the two critical sequence polymorphisms.

Discussion

In this study, we showed that the wheat *Lr34res* allele is functionally transferable into maize and confers resistance against two maize diseases of agronomic importance: the biotrophic common maize rust pathogen and the hemi-biotrophic fungus that causes northern corn leaf blight disease. Hence, *Lr34res* can be effective against biotrophic and hemi-biotrophic diseases in the same crop species. *Lr34res* has already been shown to be effective against the hemi-biotrophic fungus rice blast in rice (Krattinger *et al.*, 2016) and various biotrophic fungi such as rust and powdery mildew pathogens in wheat and barley (Risk *et al.*, 2012, 2013). Furthermore, *Lr34res* has been associated with resistance against the hemi-biotrophic fungus spot blotch (*Bipolaros sorokiniana*) in wheat (Lillemo *et al.*, 2013).

The infection processes of these fungi involve several steps, including prepenetration, penetration, colonization and sporulation (Tucker and Talbot, 2001). Each fungus uses a different mechanism to invade its host plant. Penetration can be direct,



Figure 3 Measurement of growth parameters in *Lr34res* maize plants. Plant fresh weight in kg and plant height in m was assessed for the different plants and corresponding sibs using at least three biological replicates of 14-week-old plants. Statistical analysis was performed using the Mann–Whitney–Wilcoxon test and was always P < 0.05. Errors bars represent standard errors from the three to seven biological replicates.

through the cuticle and the different layers of the cell wall, as this is the case for rice blast, NCLB and powdery mildew pathogens (Haugaard et al., 2002; Knox-Davies, 1974; Pederson et al., 2012; Wathaneeyawech et al., 2015; Zabka et al., 2008; Zhang et al., 2014). Other pathogens like the rusts use natural openings such as stomata on the leaf surface (Song et al., 2011). Once inside the leaf, the obligate biotrophic rust and mildew fungi produce specialized feeding structures called haustoria for nutrient uptake and effector secretion (Haugaard et al., 2002; Pederson et al., 2012; Petre and Kamoun, 2014; Song et al., 2011; Zabka et al., 2008). The hemi-biotrophic fungi rice blast and NCLB also develop vesicles-like structures by differentiation of primary hyphae (Kankanala et al., 2007; Muiru et al., 2008; Wilson et al., 2012) that are very similar to haustoria and that are also surrounded by the host membrane (Levy and Cohen, 1983; Wilson and Talbot, 2009). In contrast to the biotrophic pathogens, however, rice blast and NCLB switch to a necrotrophic growth phase after about two to three days (Levy and Cohen, 1983; Marcel et al., 2010). The biotrophic growth phase is a commonality among all pathogens Lr34res is effective against, and although the exact molecular function of this gene is still unknown, it is likely that the LR34res protein restricts fungal growth during this phase.

Qualitative and quantitative NCLB resistance genes have been identified in maize, for example the race-specific *Ht1* gene (Welz and Geiger, 2000) and the quantitative gene *Htn1* that confers resistance against the most prevalent NCLB races (Hurni *et al.*, 2015). *Htn1* encodes a putative wall-associated receptor-like kinase that might be involved in the perception of an apoplastic signature of *E. turcicum* (Hurni *et al.*, 2015). Common rust resistance genes have been mapped to eight different loci (Rp1,

3, 4, 5, 6, 7, 8 and 9) (Hulbert, 1997). Rp1 and Rp3 are the best characterized loci, and each consists of a cluster of genes that encode intracellular nucleotide-binding, leucine-rich repeat receptor (NLR) proteins (Smith et al., 2010; Webb et al., 2002). Gene number at the Rp1 locus differs considerably among different maize cultivars and can vary from one gene in some haplotypes to more than 50 paralogous genes in others (Smith et al., 2004). Within four maize lines (HRp1-B, HRp1-M, MH95 and B73), a total of 61 Rp1 genes, with a large diversity in the LRR region, were identified. Among them, thirty-two were transcribed and each of these genes can be distinguished based on the P. sorghi isolate to which it confers resistance (Chavan et al., 2015). An example is the *Rp1-D* gene, which has been successfully used in North America between 1985 and 2000. During autumn 1999, however, the emergence of a new Rp1-D-virulent rust population infected more than 40% of the Rp1-D 'resistant hybrids' (Pataky and Pate. 2001). This demonstrates that disease resistance based on single NLR genes is prone to breakdown because of the emergence and rapid spread of new pathogen strains. In contrast, Lr34res is a durable resistance gene with no pathogen adaptation observed so far in wheat. It is therefore possible that the resistance observed in maize against common rust and NCLB will also be durable. Hence, this gene might be useful in maize disease resistance breeding.

Orthologs of Lr34 are found in some cereals, including rice with OsABCG50 (86% amino acid identity) and sorghum with Sb01g016775 (74% amino acid identity) and Sb01g016770 (72% amino acid identity) (Krattinger et al., 2011). Members of the grass genus Tripsacum, a distant relative of Zea, also carry an Lr34 ortholog. Maize does not have an ortholog of Lr34 but can be crossed with Tripsacum. As shown by James in 1979, crosses between maize and various Tripsacum species result in pollensterile hybrids but produce kernels after backcrossing with maize. Maize × Tripsacum hybrids were already used in maize breeding programmes for the introgression of traits of interest, like fatty acid composition (Duvick et al., 2006) or rootworm resistance (Prischmann et al., 2009). The Tripsacum accessions tested in this study all had the susceptible Lr34 haplotype. However, Lr34res in wheat was the result of rare mutation events. Hence, it is possible that Lr34 orthologs of the resistant haplotype might be found in a large Tripsacum collection at low frequency. Such rare variants could be crossed into the modern maize genepool by Tripsacum \times maize hybridizations. If no resistant Lr34 variants can be found, genome editing could be used to transform the susceptible into resistant haplotype.

Interspecies gene transfer is a very powerful tool to move resistances between species and can thereby result in a broader resistance spectrum. For example, the NLR gene Rxo1 has been successfully transferred from maize, where it provides resistance against diverse bacterial pathogens, such as Xanthomonas oryzae pv. oryzicola, the causal agent of bacterial streak disease, to rice (Zhao et al., 2005). Transgenic wheat expressing the Arabidopsis EFR receptor-like kinase was more resistant against the bacterial disease Pseudomonas syringe pv. oryzae. EFR perceives the highly conserved bacterial elongation factor Ef-Tu (Schoonbeck et al., 2015). Similarly, the rice receptor-like kinase (RLK) gene Xa21 has been shown to provide Banana Xanthomonas wilt resistance in banana (Tripathi et al. 2014). The nonhost resistance NHR-linked Arabidopsis genes PING 4 (coding for a phospholipase-like protein (EARLI4-like)), PING 5 (coding for a leucine-rich repeat protein kinase) and PING 6 (coding for an ankyrin repeat family protein), known to provide pre-invasion resistance to nonadapted fungal

pathogens, have been transferred into soya bean where they confer Asian soya bean rust resistance (Langenbach *et al.*, 2016). *Lr34res* also has been successfully transferred in other crop species such as barley and rice (Krattinger *et al.*, 2016; Risk *et al.*, 2013). The fact that this gene functions across different monocots indicates that the mechanism of resistance is conserved and that the substrate transported by the ABC transporter protein is likely shared among them.

It is known that expression of resistance genes often results in lower shoot biomass and negative effects (Burdon and Thrall, 2003). *Lr34res* is also known to negatively impact plant development in barley (Chauhan *et al.*, 2015; Risk *et al.*, 2013) and in rice, where it was, however, possible to balance the fitness cost by modulating the gene expression level at seedling stage (Krattinger *et al.*, 2016). From our glasshouse experiments, even under high *Lr34res* expression levels, the development of *Lr34res*expressing maize plants was not negatively affected. Whether *Lr34res* in maize does result in a small yield penalty or not can only be determined in field experiments.

To conclude, these results are a proof of concept that *Lr34res* is functionally transferrable into the globally most produced cereal crop, maize. Hence, this resistance gene is functional in all major cereals tested. In contrast to wheat and rice, however, transgenic maize plants are already commercially grown and more than 30% of the maize area was planted with GM plants in 2014 (Lucht, 2015). Besides a transgenic approach, a detailed analysis of the *Lr34* ortholog in *Tripsacum* could offer a nontransgenic approach to establish *Lr34*-like disease resistance in maize.

Experimental procedures

Maize transformation and plant characterization

The genomic *Lr34res* construct under the wheat native promoter and terminator in the binary vector p6U (*p6U:gLr34res*; Risk *et al.*, 2012) was transformed into the maize Hi-II (A188 x B73) hybrid (Horn *et al.*, 2006) using *Agrobacterium tumefaciens*mediated transformation according to a protocol previously established (Hensel *et al.*, 2009; Van der Linde *et al.*, 2012). Fifty-five T0-plants were recovered and the identification of the *Lr34res* transformants was done by PCR on genomic DNA using the *cssfr1* marker (Lagudah *et al.*, 2009). The copy number was assessed by Southern blot using 7 µg of *Eco*RI-digested genomic DNA and a *hygromycin phosphotransferase* (Ubi-HPT) ³²Plabelled probe (Figure S1a) (Risk *et al.*, 2013). Plants were selfpollinated, resulting in T1 kernels.

Maize rust infection

Three-week-old maize seedlings were grown in Jiffy pots (diameter 8 cm) with standard soil (Classic ED 73) in a growth cabinet under diurnal conditions with 16 h light at 20 °C and 8 h dark at 18 °C. Plants were then spray-infected with a *P. sorghi* isolate that was collected from a natural glasshouse infection on maize cultivar *sweet nugget*. Plants were sprayed using an air-assisted sprayer, with a solution of urediniospores (incubated at 40 °C for five minutes to break the dormancy) and oil (Fluorinert FC-43). After inoculation, infected plants were incubated in the dark under high humidity (~95%) at 16 °C for 24 h. Then, plants were shifted to diurnal conditions (16-h light/20 °C, 8-h dark/16 °C, 70% humidity). Disease symptoms were assessed macroscopically for the presence of pustules on the leave surface and using chitin quantification 12 days after infection. Quantification of chitin was performed as described in Ayliffe *et al.* (2013). Five biological replicates of plant leaf tissue were harvested, weighed, cut into 3-cm fragments and placed into 50-mL Falcon tubes. Sufficient volume of 1 \mbox{M} KOH containing 0.1% (vol/vol) Silwet L-77 (Lehle Seeds, Round Rock, TX) was added to each tube to entirely cover the tissues. Tissues were then heated in a steam cooker for 20 min before being washed with 50 mL of 50 mM Tris, pH 7.0 and resuspended in a 50 mL volume of Tris. Plant tissues were macerated by sonication to generate a fine and uniform suspension. Two-hundred microlitre of 1 into ten water-diluted samples were added to 10 $\mbox{µL}$ of a 1-mg/mL solution of WGA-FITC before being measured using the fluorometer Synergy H1 Hybrid Reader (Biotek). The read-outs were analysed using the software Gen5, version 2.03.1 (Biotek).

NCLB cultivation and infection

Exserohilum turcicum isolate passau-1 described by Hurni *et al.*, 2015 was grown *in vitro* for three weeks on potato dextrose agar (PDA) (19.5 g/500 mL of ddH₂O) plates in the dark, upside down, at room temperature. Spores were harvested from plates by rinsing with 0.1% Tween 20 (Carl Roth) in sterile water and scraped with a spatula and filtered through a fine mesh (0.5 mm). Final density was adjusted to $4.5 * 10^4$ spores per mL, using a Neubauer counting chamber and sprayed on the three-week-old plants. Maize plants were grown for three weeks in Jiffy pots with standard soil (Classic ED 73) (Einheitserde), 15 plants in the same tray, in the glasshouse with cycles of 16 h at 20 °C with light and 8 h at 18 °C in the dark. Once the second leaf was fully expanded, the newly emerged young leaves were removed by cutting until the end of the experiment as described in Hurni *et al.* (2015).

Infected plants were kept under the same conditions but covered by plastic hoods to maintain a high humidity. First symptoms were observed eight d.a.i. and were then recorded every day for five days. Disease severity was calculated as previously described by Hurni *et al.* (2015) and Madden *et al.* (2007).

Quantitative real-time PCR analysis of Lr34res expression

Expression of *Lr34res* was quantified using RT-qPCR. Total RNA was extracted from three-week-old seedling leaves using the SV Total RNA lsolation System (Promega). RNA was quantified using a Nanodrop ND-1000 spectrophotometer, and RNA integrity was checked on a 0.8% agarose gel in 1× sodium-borate (SB) buffer. The cDNA synthesis was then performed from 500 ng of RNA using the iScriptTM advanced cDNA synthesis kit (Bio-Rad).

Expression of *Lr34res* was determined by RT-qPCR in a 10-µL volume reaction including 5 µL of KAPA SYBR[®] fast qPCR master mix (KAPA Biosystems), forward and reverse *Lr34res* qPCR primer (Risk *et al.*, 2012) (500 nm final concentration) and 4 µL of 1:20 diluted cDNA. Samples were run on a CFX96 Touch Real-time PCR (Bio-Rad). Folylpolyglutamate synthase (FPGS) was used as reference gene with the primers Forward 5' ATCTCGTTGGGG ATGTCTTG 3' Reverse 5' AGCACCGTTCAAATGTCTCC 3' (Manoli *et al.*, 2012).

Determination of growth parameters under glasshouse conditions

At least three plants per transformation event were kept until adult stage (14 weeks) in a glasshouse under LED light. The glasshouse conditions were 60% humidity and 25 °C. Plants were grown in standard soil (Classic ED 73) (Einheitserde). Fourteen-week-old plants were cut at soil level to determine fresh weight and plant height to the apex. Tassels were cut at their base and therefore excluded from the measure.

Identification of *Lr34* orthologous genes in maize and *Tripsacum*

PCR was performed on genomic DNA using primer set *Lr34_ conserved_f2* (5'-CCATGGCCCTCGAAATGAAG-3') and *Lr34_ conserved_r2* (5'-GCTGGTATTGCATATGCCCA-3').

PCRs were performed in a 20- μ L total volume using 1 μ L of 40 ng/ μ L gDNA and 19 μ L of PCR mix (Sigma buffer 1× (Sigma-Aldrich), dNTPs (0.125 mM), primer forward and reverse (0.5 mM) and Taq polymerase (Sigma–Aldrich) (0.25 μ L of 5 U/ μ L)).

PCR products (~755 bp) were purified using the GenElute Gel extraction kit (Sigma–Aldrich). Purified products were sequenced using 1 μ L of purified PCR product, 0.80 μ L Big Dye/BrightDye Terminator Mix (Thermo-Fisher), 0.25 μ L primer (5 pmol/ μ L) and 2 μ L of 5× sequencing buffer in a total reaction volume of 10 μ L. Sequencing was performed using an ABI-3730 Sanger sequencer (Applied Biosystems) and sequences were analysed with FinchTV[®] software (Geospiza).

Sequence alignments and phylogenetic tree construction were performed using the web service phylogeny.fr (Dereeper *et al.*, 2008, 2010). The multiple alignment was performed with MUSCLE[®] software (Edgar, 2004), the alignment curation with GBlock[®] software (Castresana, 2000; Talavera and Castresana, 2007), the construction of the phylogenetic tree with PhyML[®] (Guindon *et al.*, 2010) and the tree rendering with TreeDyn[®] (Chevenet *et al.*, 2006).

Acknowledgement

The authors would like to thank Dr. Harald Keunecke from KWS, Einbeck, Germany, for providing the common rust (*Puccinia sorghi*) isolate. We also want to acknowledge Dr. Susanne Dreisigacker from CIMMYT, Mexico, for providing leaf material of the different *Tripsacum* species. We thank Thomas Widiez and Jérôme Laplaige from ENS Lyon, France, for the *Lr34* maize propagation. We are thankful to Petra Hoffmeister, from IPK Gatersleben, as well as Geri Herren and Linda Luethi, from UZH Switzerland, for their valuable technical support. The work was supported by an advanced investigator grant of the European Research Concil (ERC-2009-AdG 249996, Durableresistance) and the Swiss National Science Foundation grant 31003B-144081/1. S.G.K. is supported by an Ambizione grant of the Swiss National Science Foundation.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

References

- Ayliffe, M., Periyannan, S.K., Feechan, A., Dry, I., Schumann, U., Wang, M.B., Pryor, A. et al. (2013) A simple method for comparing fungal biomass in infected plant tissues. *Mol. Plant Microbe Interact.* **26**, 658–667.
- Bettgenhaeuser, J., Gilbert, B., Ayliffe, M. and Moscou, M.J. (2014) Nonhost resistance to rust pathogens a continuation of continua. *Front. Plant Sci.* **5**, 664.
- Brunner, S., Stirnweis, D., Diaz Quijano, C., Buesing, G., Herren, G., Parlange, F., Barret, P. *et al.* (2012) Transgenic *Pm3* multilines of wheat show

increased powdery mildew resistance in the field. Plant Biotech. $\mathbf{10},\ \mathbf{398-409}.$

- Burdon, J.J. and Thrall, P.H. (2003) The fitness costs to plants of resistance to pathogens. *Genome Biol.* **4**, 227.
- Castresana, J. (2000) Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol.* **4**, 540–552.
- Chaudhary, D.P., Kumar, S. and Singh, S. (2014) Maize: Nutrition Dynamics and Novel Uses. Springer India, New Delhi doi: 10.1007/978-81-322-1623-0.
- Chauhan, H., Boni, R., Bucher, R., Kuhn, B., Buchmann, G., Sucher, J., Selter, L.L. et al. (2015) The wheat resistance gene Lr34 results in the constitutive induction of multiple defense pathways in transgenic barley. Plant J. 84, 202– 215.
- Chavan, S., Gray, J. and Smith, S.M. (2015) Diversity and evolution of *Rp1* rust resistance genes in four maize lines. *Theor. Appl. Genet.* **128**, 985–998.
- Chevenet, F., Brun, C., Banuls, A.-L., Jacq, B. and Christen, R. (2006) TreeDyn: towards dynamic graphics and annotations for analyses of trees. *BMC Bioinformatics*, 7, 439.
- Dakouri, A., McCallum, B.D., Walichnowski, A.Z. and Cloutier, S. (2010) Finemapping of the leaf rust *Lr34* locus in Triticum aestivum (L.) and characterization of large germplasm collections support the ABC transporter as essential for gene function. *Theor. Appl. Genet.* **121**, 373–384.
- Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.F. *et al.* (2008) Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res.* **36**, W465–W469.
- Dereeper, A., Audic, S., Claverie, J.-M. and Blanc, G. (2010) G. BLAST-EXPLORER helps you building datasets for phylogenetic analysis. *BMC Evol. Biol.* **12**, 8.
- Duvick, S.A., Pollak, L.M., Edwards, J.W. and White, P.J. (2006) Altering the fatty acid composition of Corn Belt corn through Tripsacum introgression. *Maydica*, **51**, 409–416.
- Eckardt, N.A. (2006) Identification of rust fungi avirulence elicitors. *Plant Cell*, **18**, 1–3.
- Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **32**, 1792–1797.
- Ellis, J.G., Lagudah, E.S., Spielmeyer, W. and Dodds, P.N. (2014) The past, present and future of breeding rust resistant wheat. *Front. Plant Sci.* 5, 641. FAO (2016) access 06/2016, http://faostat3.fao.org/home/E
- Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W. and Gascuel, O. (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. System. Biol. 59, 307– 321.
- Haugaard, H., Collinge, B.D. and Lyngkjær, M.F. (2002) Mechanisms involved in control of Blumeria graminis f.sp. hordei in barley treated with mycelial extracts from cultured fungi. *Plant. Pathol.* **51**, 612–620.
- Hensel, G., Kastner, C., Oleszczuk, S., Riechen, J. and Kumlehn, J. (2009) Agrobacterium-mediated transformation of cereal crop plants: current protocols for barley, wheat, triticale and maize. *Int. J. Plant Genom.* 2009 Article 835608, doi: 10.1155/2009/835608.
- Horn, M.E., Harkey, R.L., Vinas, A.K., Drees, C.F., Barker, D.K. and Lane, J.R. (2006) Use of Hi II-Elite inbred hybrid in agrobacterium-based transformation of maize. *In Vitro Cell Dev-PL*, **42**, 359–366.
- Hulbert, S.H. (1997) Structure and evolution of the rp1 complex conferring rust resistance in maize. *Annu. Rev. Phytopathol.* **35**, 293–310.
- Hurni, S., Scheuermann, D., Krattinger, S.G., Kessel, B., Wicker, T., Herren, G., Fitze, M. et al. (2015) The maize disease resistance gene *Htn1* against northern corn leaf blight encodes a wall-associated receptor-like kinase. *Proc. Natl. Acad. Sci. USA*, **112**, 8780–8785.
- Jasinski, M., Ducos, E., Martinoia, E. and Boutry, M. (2003) The ATP-binding cassette transporters: structure, function, and gene family comparison between rice and Arabidopsis. *Plant Physiol.* **131**, 1169–1177.
- Kankanala, P., Czymmek, K. and Valent, B. (2007) Roles for rice membrane dynamics and plasmodesmata during biotrophic invasion by the blast fungus. *Plant Cell*, **19**, 706–724.
- Knox-Davies, P.S. (1974) Penetration of maize leaves by *Helminthosporium turcicum*. *Phyto*, **64**, 1468.
- Kolmer, J.A., Singh, R.P. and Garvin, D.F. (2008) Analysis of the *Lr34/Yr18* rust resistance region in wheat germplasm. *Crop Sci.* **48**, 1841–1852.

- Krattinger, S.G. and Keller, B. (2016) Trapping the intruder immune receptor domain fusions provide new molecular leads for improving disease resistance in plants. *Genome Biol.* **19**, 23.
- Krattinger, S.G., Lagudah, E.S., Spielmeyer, W., Singh, R.P., Huerta-Espino, J., McFadden, H., Bossolini, E. *et al.* (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science* **323**, 1360–1363.
- Krattinger, S.G., Lagudah, E.S., Wicker, T., Risk, J.M., Ashton, A.R., Selter, L.L., Matsumoto, T. *et al.* (2011) *Lr34* multi-pathogen resistance ABC transporter: molecular analysis of homoeologous and orthologous genes in hexaploid wheat and other grass species. *Plant J* **65**, 392–403.
- Krattinger, S.G., Jordan, D.R., Mace, E.S., Raghavan, C., Luo, M.C., Keller, B. and Lagudah, E.S. (2013) Recent emergence of the wheat *Lr34* multipathogen resistance: insights from haplotype analysis in wheat, rice, sorghum and *Aegilops tauschii*. *Theor. Appl. Genet.* **126**, 663–672.
- Krattinger, S.G., Sucher, J., Selter, L.L., Chauhan, H., Zhou, B., Tang, M., Upadhyaya, N.M. *et al.* (2016) The wheat durable, multipathogen resistance gene *Lr34* confers partial blast resistance in rice. *Plant Biotechnol. J.* **14**, 1261–1268.
- Lagudah, E.S., Krattinger, S.G., Herrera-Foessel, S., Singh, R.P., Huerta-Espino, J., Spielmeyer, W., Brown Guedira, G. *et al.* (2009) Gene-specific markers for the wheat gene Lr34/Yr18/Pm38 which confers resistance to multiple fungal pathogens. *Theor. Appl. Genet.* **119**, 889–898.
- de Lange, E.S., Balmer, D., Mauch-Mani, B. and Turlings, T.C.J. (2014) Insect and pathogen attack and resistance in maize and its wild ancestors, the teosintes. *New Phytol.* **204**, 329–341.
- Langenbach, C., Schultheiss, H., Rosendahl, M., Tresch, N., Conrath, U. and Goellner, K. (2016) Interspecies gene transfer provides soybean resistance to a fungal pathogen. *Plant Biotechnol. J.* **14**, 699–708.
- Levy, Y. and Cohen, Y. (1983) Biotic and environmental factors affecting infection of sweet corn with *Exserohilum turcicum*. *Am. Phytopathol. Soc.* **73**, 722–725.
- Lillemo, M., Joshi, A.K., Prasad, R., Chand, R. and Singh, R.P. (2013) QTL for spot blotch resistance in bread wheat line Saar co-locate to the biotrophic disease resistance loci *Lr34* and *Lr46. Theor. Appl. Genet.* **126**, 711–719.
- Lucht, J.M. (2015) Public acceptance of plant biotechnology and GM crops. *Viruses*, **7**, 4254–4281.
- Madden, L.V., Hughes, G. and van den Bosch, F. (2007) The Study of Plant Disease Epidemics. Minnesota: The American Phytopathological Society, APS Press St. Paul.
- Manoli, A., Sturaro, A., Trevisan, S., Quaggiotti, S. and Nonis, A. (2012) Evaluation of candidate reference genes for qPCR in maize. *J. Plant Physiol.* 169, 807–815.
- Marcel, S., Sawers, R., Oakeley, E., Angliker, H. and Paszkowski, U. (2010) Tissue-adapted invasion strategies of the rice blast fungus *Magnaporthe* oryzae. Plant Cell, **22**, 3177–3187.
- McDonald, B.A. and Linde, C. (2002) The population genetics of plant pathogens and breeding strategies for durable resistance. *Euphytica*, **124**, 163–180.
- Moore, J.W., Herrera-Foessel, S., Lan, C., Schnippenkoetter, W., Ayliffe, M., Huerta-Espino, J., Lillemo, M. *et al.* (2015) A recently evolved hexose transporter variant confers resistance to multiple pathogens in wheat. *Nat. Genet.* **47**, 1494–1498.
- Muiru, W.M., Mutitu, E.W., Kimenju, J.W., Koopmann, B.Ans. and Tiedemann, A.V. (2008) Infectious structures and response of maize plants to invasion by *Exserohilum turcicum* (Pass). in compatible and incompatible host pathogen systems. J. Appl. Biosci. **10**, 532–537.
- Munkvold, G.P. and White, D.G. (2016) *Compendium of Corn Diseases*, 4th edn. St. Paul, Minnesota, U.S.A.: APS Press ISBN 978-0-89054-492-1.
- Oerke, E.C. (2006) Crop losses to pests. J. Agric. Sci. 144, 31-43.
- Pataky, J.K. and Pate, M.C. (2001) Resistance genes in the *Rp1* region of maize effective against Puccinia sorghi virulent on the *Rp1-D* gene in north America. *Am. Phytopatol. Soc.* **2**, 165–168, D-2000-1120-02R.
- Pederson, C., van Themaat, E.v.L., McGuffin, L.J., Abbott, J.C., Burgis, T.A., Barton, G., Bindschedler, L.V. *et al.* (2012) Structure and evolution of barley powdery mildew effector candidates. *BMC Genom.* **13**, 694.
- Petre, B. and Kamoun, S. (2014) How do filamentous pathogens deliver effector proteins into plant cells? *PLoS Biol.* **12**, e1001801.

496 Justine Sucher et al.

- Prischmann, D.A., Dashiell, K.E., Schneider, D.J. and Eubanks, M.W. (2009) Evaluating Tripsacum-introgressed maize germplasm after infestation with western corn rootworms (Coleoptera: Chrysomelidae). J. Appl. Entomol. 133, 10–20.
- Rea, P.A. (2007) Plant ATP-binding cassette transporters. *Annu. Rev. Plant Biol.* 58, 347–375.
- Risk, J.M., Selter, L.L., Krattinger, S.G., Viccars, L.A., Richardson, T.M., Buesing, G., Herren, G. et al. (2012) Functional variability of the Lr34 durable resistance gene in transgenic wheat. Plant Biotechnol. J. 10, 477–487.
- Risk, J.M., Selter, L.L., Chauhan, H., Krattinger, S.G., Kumlehn, J., Hensel, G., Viccars, L.A. et al. (2013) The wheat Lr34 gene provides resistance against multiple fungal pathogens in barley. *Plant Biotechnol. J.* **11**, 847–854.
- Schoonbeck, H.J., Wang, H.H., Stefanato, F.L., Craze, M., Bowden, S., Wallington, E., Zipfel, C. et al. (2015) Arabidopsis EF-Tu receptor enhances bacterial disease resistance in transgenic wheat. New Phytol. 206, 606–613.
- Singh, R.P. and Huerta-Espino, J. (1997) Effect of leaf rust resistance gene *Lr34* on grain yield and agronomic traits of spring wheat. *Crop Sci.* **37**, 390–395.
- Singh, R.P., Payne, T.S. and Rajaram, S. (1991) Characterization of variability and relationships among components of partial resistance to leaf rust in CIMMYT bread wheats. *Theor. Appl. Genet.* **82**, 674–680.
- Singh, R.P., Huerta-Espino, J. and William, H.M. (2005) Genetics and breeding for durable resistance to leaf and stripe rusts in wheat. *Turk J. Agric.* 29, 121– 127.
- Singla, J. and Krattinger, S. (2016) Biotic stress resistance genes in wheat. Encyclopedia of Food Grains. Academic Press: Oxford 388–392 doi: 10.1016/ B978-0-12-394437-5.00229-1.
- Smith, S.M., Pryor, A.J. and Hulbert, S.H. (2004) Allelic and haplotypic diversity at the *rp1* rust resistance locus of maize. *Genetics*, **167**, 1939–1947.
- Smith, S.M., Steinau, M., Trick, H.N. and Hulbert, S.H. (2010) Recombinant *Rp1* genes confer necrotic or nonspecific resistance phenotypes. *Mol. Genet. Genom.* 283, 591–602.
- Song, X., Rampitsch, C., Soltani, B., Mauthe, W., Linning, R., Banks, T., McCallum, B. et al. (2011) Proteome analysis of wheat leaf rust fungus, *Puccinia triticina*, infection structures enriched for haustoria. *Proteomics*, **11**, 944–963.
- Spielmeyer, W., Mago, R., Wellings, C. and Ayliffe, M. (2013) Lr67 and Lr34 rust resistance genes have much in common–they confer broad spectrum resistance to multiple pathogens in wheat. BMC Plant Biol. 13, 96.
- Talavera, G. and Castresana, J. (2007) Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.* 56, 564–577.
- Tripathi, J.N., Lorenzen, J., Bahar, O., Ronald, P. and Tripathi, L. (2014) Transgenic expression of the rice Xa21 pattern-recognition receptor in banana (Musa sp.) confers resistance to Xanthomonas campestris pv. musacearum. Plant Biotechnol. J. 12, 663–673.
- Tucker, S.L. and Talbot, N.J. (2001) Surface attachment and pre-penetration stage development by plant pathogenic fungi. *Annu. Rev. Phytopathol.* **39**, 385–417.
- Van der Linde, K., Hemetsberger, C., Kastner, C., Kaschani, F., van der Hoorn, R.A.L., Kumlehn, J. and Doehlemann, G. (2012) A maize cystatin suppresses host immunity by inhibition of apoplastic cysteine proteases. *Plant Cell*, **24**, 1285–1300.
- Wathaneeyawech, S., Sirithunya, P. and Smitamana, P. (2015) Study of the host range of northern corn leaf blight disease and effect of *Exserohilum turcicum* toxin on sweet corn. *J. Agric. Technol.* **11**, 953–963.
- Webb, C.A., Richter, T.E., Collins, N.C., Nicolas, M., Trick, H.N., Pryor, T. and Hulbert, S.H. (2002) Genetic and molecular characterization of the maize *rp3* rust resistance locus. *Genetics*, **162**, 381–394.
- Welz, H.G. and Geiger, H.H. (2000) Genes for resistance to northern corn leaf blight in diverse maize populations. *Plant Breeding*, **119**, 1–4.

- Wilson, R.A. and Talbot, N.J. (2009) Under pressure: investigating the biology of plant Infection by *Magnaporthe oryza. Nat. Rev.: Microbiol.* 7, 185– 195.
- Wilson, R.A., Fernandez, J., Quispe, C.F., Gradnigo, J., Seng, A., Moriyama, E. and Wright, J.D. (2012) Towards defining nutrient conditions encountered by the rice blast fungus during host Infection. *PLoS ONE*, 7, e47392.
- Wisser, R.J., Balint-Kurti, P.J. and Nelson, R.J. (2006) The genetic architecture of disease resistance in maize: a synthesis of published studies. *Phytopathology*, 96, 120–129.
- Zabka, V., Stangl, M., Bringmann, G., Vogg, G., Riederer, M. and Hildebrandt, U. (2008) Host surface properties affect pre-penetration processes in the barley powdery mildew fungus. *New Phytol.* **177**, 251–263.
- Zhang, H., Wu, Z., Wang, C., Li, Y. and Xu, J.-R. (2014) Germination and infectivity of microconidia in the rice blast fungus *Magnaporthe oryzae*. *Nat. Commun.* 5, 4518.
- Zhao, B., Lin, X., Poland, J., Trick, H., Leach, J. and Hulbert, S. (2005) A maize resistance gene functions against bacterial streak disease in rice. *Proc. Natl. Acad. Sci. USA*, **102**, 15383–15388.

Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Transgene copy number detection. (a) Schematic representation of the *Lr34res* construct. (b) Southern blot showing T-DNA copy number in the *Lr34res* transgenic maize. Lines 161, 163 and 164 at T2 generation are represented. + indicates plants with *Lr34res* and – segregating sib lines without *Lr34res*.

Figure S2. Macroscopic common rust symptoms on plants derived from events 161 and 163 and corresponding sibs, 12 d.a.i. Infections were done at seedling stage on three-week-old plants.

Figure S3. Macroscopic observation of NCLB symptoms on the different *Lr34res* transgenic maize plants and their corresponding sibs 14 days after infection. Infections were done at seedling stage on three-week-old plants. Scale bar = 10 mm.

Figure S4. Study of orthologous Lr34 genes in maize and its distant relatives of the genus Tripsacum. (a) Agarose gel showing genomic DNA amplification of Lr34 on wheat (Chinese-Spring), OsABCG50 on rice (Nipponbare) and the Lr34 orthologs on 10 different Tripsacum species. No amplification was obtained for maize Hi-II. (b) Phylogenetic tree based on genomic DNA sequences of Lr34, of the most homologous maize gene sequence (GRMZM2G014282), of three of the most homologous rice genes sequences (OsABCG50, OsABCG41 and OsABCG49), two sorghum orthologous sequences (Sb01 g016775 and Sb01 g016700), one Brachypodium distachyon homolog (Bradi4 g45397), and 5 different Tripsacum species. The "Lr34 orthologous cluster" is marked in red. The sequence from Penicillium chrysogenum (Pc12 g09900) was used as outgroup to root the tree (Krattinger et al., 2011). Numbers indicate how many times the sequences to the right of the fork occurred in the same group out of 100 trees.

Table S1. List of the 13 *Tripsacum* accessions used for the study of the *Lr34* orthologous gene. ID number corresponds to the reference in the CIMMYT maize germplasm database.