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Review

Research review

Exploring and exploiting the boundaries of host specificity using the cereal rust and mildew models

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Summary

Individual plants encounter a vast number of microbes including bacteria, viruses, fungi and oomycetes through their growth cycle, yet few of these pathogens are able to infect them. Plant species have diverged over millions of years, co-evolving with few specific pathogens. The host boundaries of most pathogen species can be clearly defined. In general, the greater the genetic divergence from the preferred host, the less likely that pathogen would be able to infect that plant species. Co-evolution and divergence also occur within pathogen species, leading to highly specialized subspecies with narrow host ranges. For example, cereal rust and mildew pathogens (*Puccinia* and *Blumeria* spp.) display high host specificity as a result of ongoing co-evolution with a narrow range of grass species. In rare cases, however, some plant species are in a transition from host to nonhost or are intermediate hosts (near nonhost). Barley was reported as a useful model for genetic and molecular studies of nonhost resistance due to rare susceptibility to numerous heterologous rust and mildew fungi. This review evaluates host specificity in numerous *Puccinia*/*Blumeria*–cereal pathosystems and discusses various approaches for transferring nonhost resistance (NHR) genes between crop species to reduce the impact of important diseases in food production.

Introduction

Rust and mildew fungi are widely distributed, highly specialized, obligate biotrophic pathogens that infect many vascular plant species. To complete their life cycle, both must feed on living tissues of host plants to obtain a source of carbon, and in doing so reduce host fitness (Price, 1987; Plummer *et al.*, 1990; Roelfs *et al.*, 1992). Although several rust pathogen species have been cultured artificially, they grow very poorly and are consequently considered obligate biotrophs (Maclean *et al.*, 1971; Wolf, 1974). Of great significance in agriculture are species of the genera *Puccinia* and *Blumeria*, which infect (able to colonize, produce haustoria and sporulate) many important cereal species (sorghum, cereal rye, wheat, maize, oats and barley) within the Poaceae family. Of the 'Top 10' economically and scientifically important plant pathogens, *Puccinia* spp. ranked 3rd, *Blumeria graminis* 6th, the flax rust pathogen (*Melamspora lini*) 10th, with the Asian soybean rust pathogen (*Phakopsora pachyrhizi*) narrowly missing out (Dean *et al.*, 2012).

Among the *Puccinia* species, those that attack wheat are considered to be most important. Stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), can cause crop failure during heavy epidemic years, whilst stripe rust, *P. striiformis* f. sp. *tritici* (*Pst*), and leaf rust, *P. triticina* (*Pt*), can cause losses of up to 50% on susceptible varieties. For *Blumeria graminis,* the forms adapted to either wheat (f. sp. *tritici*) or barley (f. sp. *hordei*) cause the most economically significant diseases. Resistance, although more cost-effective and environmentally acceptable than fungicide use, is vulnerable to breakdown due to genomic plasticity and rapid evolution in pathogen populations (Bradley, 1962; Anikster & Wahl, 1979). The emergence of *Pgt* race TTKSK (Ug99) provided a graphic reminder of how rust pathogens can acquire virulence for widely important rust resistance genes such as stem rust resistance gene *Sr31* (Pretorius *et al.*, 2000, 2010; Wanyera *et al.*, 2006; Singh *et al.*, 2011). Transferring *R* genes that confer nonhost resistance (NHR) between closely related species is one strategy that could expand the arsenal at our disposal to control biotrophic diseases of cereal crops. Although poorly understood relative to host resistance, histological, genetic and functional studies investigating resistance to heterologous mildew and rust pathogens mainly in barley suggest the involvement of both pre- and post-haustorial mechanisms (Fig. 1). The *mlo* resistance provides durable broad-spectrum penetration resistance to all *formae speciales* ('special forms'; f. spp.) of *Blumeria graminis*, and has been used widely and remained effective for decades (Schwarzbach, 1979; Jørgensen, 1992).

Cereal rust and mildew pathogen species have been subdivided into *formae speciales* based on the range of grasses they commonly infect. Despite this, the genetic similarity between different *formae speciales* closely mirrors that of their preferred

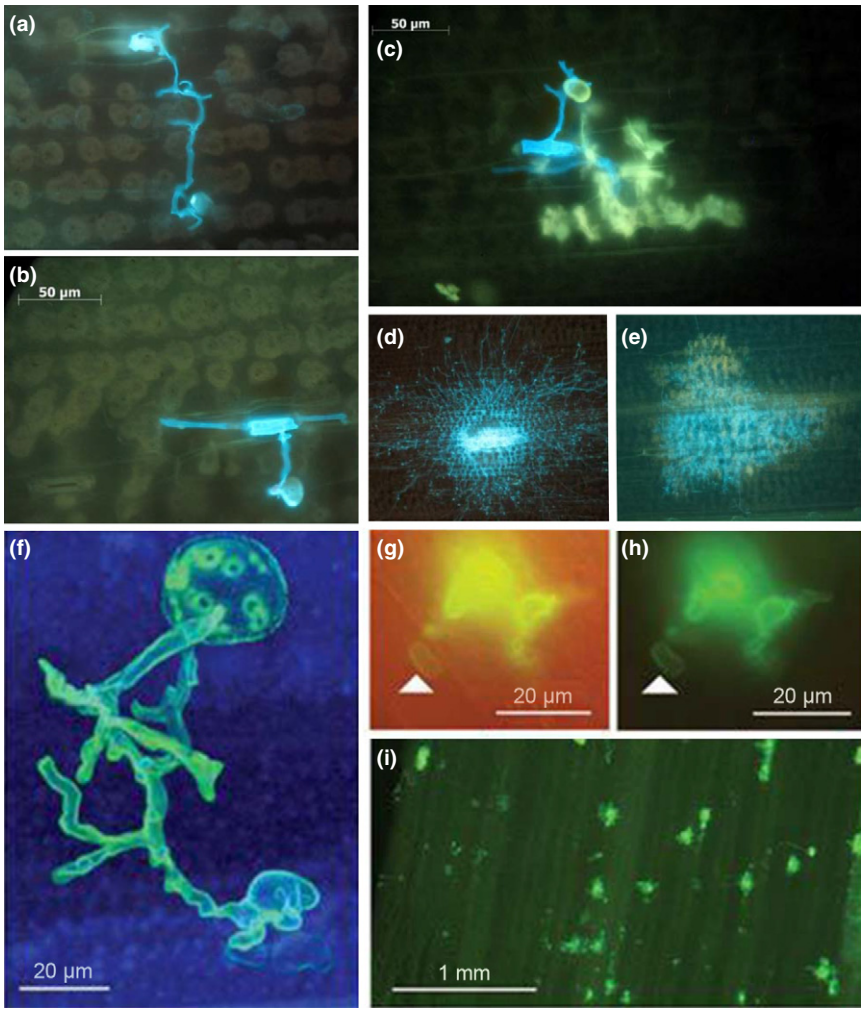

Fig. 1 Histological analysis of infection structures of the heterologous cereal rust pathogens *Puccinia graminis* f. sp. *avenae* (causal agent of oat stem rust) on barley and oat, and *Puccinia hordei* (causal agent of barley leaf rust) on rice. (a) Nonpenetrating infection unit of *Puccinia graminis* f. sp. *avenae*–Evertsholm isolate on Alfred oat.
(b) Early abortion without necrosis on Vada. (c) Early abortion with necrosis on barley line with susceptibility to *P. triticina* (SusPtrit).
(d) Established colony without necrosis on Cebeco oat. (e) Established colony with necrosis on SusPtrit. (f) Confocal microscopy showing infection structures of a *P. hordei* infections on rice. The spore, germ tube and appressorium are present on the surface of the leaf, whereas the underlying substomatal vesicle is inside the leaf and seen as a paler structure at the bottom right of the panel.
(g) Haustorium produced by a *P. hordei* infection site on rice. (h) The same infection site shown in (g), demonstrating the epidermal cell location of the *P. hordei* haustorium (i). variation in *P. hordei* infection site sizes observed on a single rice leaf, white arrowheads indicate the position of haustoria. White arrowheads indicate the position of haustoria. Adapted, with permission, from Ayliffe *et al.* (2011) © 2011 The American Phytopathological Society and Dracatos *et al.* (2016). © 2016 The American Phytopathological Society.

grass hosts, implying the presence of shared effectors in the pathogen and *R* genes in the respective hosts. In some instances, however, a host plant may evolve at a faster rate than the specialized pathogen, thus acquiring nonhost status or be in transition between host and nonhost. This review discusses recent advances in determining host–pathogen boundaries in cereal pathosystems and the possible utilization of NHR in an agricultural context.

Mechanisms of host and nonhost resistance in plants

Most plants are resistant to most diseases, implying that susceptibility is more of an exception rather than the rule. The preformed physical and chemical barriers are generally conserved between closely related grass/cereal species. By contrast, leaf surface topography and chemical cues are likely to differ significantly between more distantly related plant species (i.e. between the leaves of wheat and *Arabidopsis*). The initial inability of a pathogen to locate stomata due to the surface topography of foreign leaf surfaces is likely to be a common contributor to NHR. However, this does not account for the inability of some *Puccinia* taxa to infect particular cereal grasses. In general, heterologous rust fungi that are pathogenic on particular graminaceous host species have the ability to find and penetrate stomata on nonhost grasses and cereal species (Niks, 1986). Although there may be variations in the initial chemical cues, both adapted and heterologous cereal

rust pathogens are equally likely to navigate the topography of a monocot plant species, find stomata and produce appressoria. However, they are normally unable to form haustoria (Niks & Rubiales 2002; Niks, 1986).

The status of a plant species as either a host or a nonhost is not always clear cut. Bettgenhaeuser *et al.* (2014) described the boundaries between host and nonhost as more of a continuum, referring to a fluidity between the ability of closely related pathogens to infect closely related grass species rather than the clear distinction between host and nonhost. From the plant perspective, both host and NHR are expressed as an immune response. Similar to mammalian systems, the first line of defence in plants is the innate immune response that is governed by the recognition of conserved molecular signatures (i.e. chitin) known as pathogen-associated molecular patterns (PAMPs) by plant receptors known as pattern recognition receptors (PRRs) (Glazebrook, 2005; Jones & Dangl, 2006; Zipfel, 2008). This very early detection response of nonself in plants is known as PAMP-triggered immunity (PTI) and is best characterized in model organisms such as rice and Arabidopsis (Meyers *et al.*, 2003; Zhou *et al.*, 2004; Chinchilla *et al.*, 2006; Zipfel, 2008; Ayliffe *et al.*, 2011). Pre-haustorial resistance (PHR) is where fungal growth is terminated before plant cell entry and has proven more durable against biotrophic pathogens (Rubiales & Niks, 1996; Martínez *et al.*, 2001; Serfling *et al.*, 2016). PHR led to macroscopic immunity in previous studies in wheat (Anker & Niks, 2001; Serfling *et al.*, 2016) and barley (Hoogkamp *et al.*, 1998; Dracatos *et al.*, 2014, 2016), and is a feature of host, near nonhost and nonhost interactions. The best and most widely studied examples of such resistance are mutant or naturally defective alleles of the *Mlo* locus in barley (Tosa & Shishiyama, 1984). PHR is commonly associated with formation of cell wall reinforcements known as papillae (Collins *et al.,* 2007; Heath, 2002; Niks, 1986). An effective physical barrier to infection is achieved through cell wall-associated defences, including the deposition of substances such as silica (Heath *et al.,* 1992) and lignin as peroxidative cross-linking of phenolic compounds (Lamb & Dixon, 1997). It may be assumed that behind PHR may be the presence of induced resistance (redundant *R* genes) that may only be expressed following post-haustorial stages of infection.

Plant pathogens have also co-evolved with their respective host species, acquiring specific virulence mechanisms and effector molecules that are used to re-program the host cell and suppress PTI, allowing the pathogen to acquire sugars to support its growth and sporulation. The ability of would-be pathogens to suppress PTI and cause disease is dependent upon the array of effectors virulence factors in the pathogen and PRRs in the plant genotype, respectively. Closely related pathogens share common effectors and the proportion of shared effectors is likely to increase depending on the extent of relatedness and hence is negatively correlated with taxonomic distance (Schulze-Lefert & Panstruga, 2011). For example, different *formae speciales* of the same rust pathogen species (i.e. *P. striiformis* f. sp. *tritici* vs f. sp. *hordei*) would be expected to share more effectors compared to different *Puccinia* species (i.e. *P. striiformis* vs *P. triticina*), and especially pathogens of different genera (i.e. *Puccinia* vs *Blumeria*). A minor subset of effectors are classic avirulence genes, which are involved in eliciting receptors that trigger a defence response to constrain pathogen spread (effector-triggered immunity, ETI).

As found in the flax–flax rust pathosystem (Ellis *et al.*, 2007), *Puccinia* and *Blumeria* spp are believed to secrete effectors that blunt basal resistance targets. Some of these effectors are directly recognized by NLR (nucleotide-binding domain leucine-rich repeat containing) receptor proteins or indirectly by the effect they have on their guard proteins. Although well established for bacterial resistance very little is known for both the guard and decoy models in plant fungal interactions (van der Hoorn & Kamoun, 2008). It is well documented, however, that pathogen avirulence (*Avr*) gene effector products are recognized by the LRR domain of NLR proteins, and the generation of polymorphisms in either pathogen *Avr* or host *R* genes (gene-for-gene) via well-characterized evolutionary mechanisms are critical for pathogen survival (Upadhyaya *et al.*, 2014). Recent studies on the isolation of rust resistance genes expressed in wheat during ETI suggest that in all cases NLR genes encoding cytoplasmic receptor proteins were responsible for the observed resistance (Periyannan *et al.*, 2013; Saintenac *et al.*, 2013; Mago *et al.*, 2015; Steuernagel *et al.*, 2015; Sánchez-Martín *et al.*, 2016; Chen *et al.*, 2017; Salcedo *et al.*, 2017). Fewer rust resistance genes have been isolated in barley relative to wheat. The two barley stem rust resistance genes, *Rpg1* and *Rpg5*, have been cloned and neither encodes typical NLR type proteins (*Rpg1*-Serine/Threonine Kinase, *Rpg5*-NBS-LRR-Serine/Threonine Kinase) (Brueggeman *et al.*, 2002, 2008). Whether this observation is due to chance or because resistance in barley is mediated largely by different mechanisms, remains unclear. As illustrated in a very recent review by Uauy *et al.* (2017), a higher number of naturally occurring recessive genes exist in functional diploid crops such as barley that may otherwise be masked in polyploid crops such as wheat due to redundancy between the homeologous gene copies. It was therefore hypothesized that over time humans have selected for dominant and semi-dominant traits in wheat, such as NLRs involved in hypersensitive resistance relative to barley, where knockout recessive mutations are more prevalent. However, given the ancestoral relationship between *Triticum* and *Hordeum* spp. in the Triticeae subtribe and previous knowledge of the evolution of disease resistance in plants, it is likely that NLRs also play a significant role in rust resistance in barley to rust diseases.

According to Schulze-Lefert & Panstruga (2011), ETI is not just limited to adapted pathogen recognition; it can also play an important role in NHR, particularly against pathogens that are adapted to plant species closely related to the nonhost species. From an evolutionary point of view, the similarity and abundance (1% of coding genes) of NLRs across grass genomes possibly suggests not only a 'birth and death role' for this gene family, but also a role in NHR (Michelmore & Meyers, 1998). A recent example, albeit not in grasses, was a study reporting on the isolation of an NLR from pigeon pea (closely related to soybean) effective against the Asian soybean rust pathogen (*Phakopsora pachyrhizi*) (Kawashima *et al.*, 2016). The case above implies that the *P. pachyrhizi* isolate carried an avirulence gene,

the product of which elicited an ETI response in the pigeon pea accession used.

The evolution of host specificity – pathogens and grass species

Puccinia spp. that cause rusts on monocot grasses are good examples of pathogen species that can evolve rapidly due their modes of reproduction, method of transmission and ability to mutate. *Puccinia graminis* can infect 365 species across 54 genera (Anikster, 1984). Some rust (*P. graminis* and *P. striiformis*) and mildew (*Blumeria graminis*) pathogen species have further specialized due to co-evolution with different grass hosts, and thus comprise *formae speciales* that are morphologically indistinguishable or very similar but are specialized to different host species. The concept of *formae specialis* was first introduced by Eriksson & Henning (1896), who inoculated a range of grass species with different isolates from different host species and identified variation within rust pathogen species in their ability to infect different grass hosts and the range of host species that could be infected. Eriksson & Henning's work essentially defined the boundaries of host range for rust pathogens such as *P. graminis* and *P. striiformis*. Recent studies reporting on the host status of various grass species to different *Puccinia* spp. identified unexpected susceptibility, potentially challenging the original boundaries of host specificity stipulated by Eriksson & Henning (1896). For example, the model grass *Brachypodium* is more receptive to both the *avenae* and *lolii formae speciales* of *P. graminis* than the *tritici* form (Figueroa *et al.*, 2013). Jin *et al.* (1993) identified wheat accessions that were susceptible to the barley crown rust pathogen (*P. coronata* var. *hordei*), despite wheat not having an adapted crown rust pathogen. Therefore, the ability of a heterologous pathogen to infect a nonhost plant is not always strictly correlated with the taxonomic distance between host and nonhost plant or pathogen. The host status of the different *Puccinia* spp adapted to both cereal and forage grasses in the Poaceae family is summarized in Fig. 2.

Different isolates of the same species derived from geographically diverse pathogen populations often differ in their virulence/avirulence spectra, influencing not only the host but also other auxiliary grass/crop accessions they are able to colonize. For example, in Australia two founder populations of *P. striiformis* f. sp. *tritici* were derived from separate exotic incursions and both differ significantly in their adaptation to temperature (Loladze *et al.*, 2014), host specificity and the wheat varieties they can infect (Wellings, 2007). Since each incursion, both founder populations systematically evolved virulence for most of the all-stage resistance genes deployed in Australian wheats. However, such isolate specificity also extends to other plant species that are closely related to wheat. The US-derived 134 E16 A+ pathotype evolved virulence for rye-derived resistance genes relative to the European-derived pathotype 104 E137 A-, resulting in adaptation to Triticale varieties including Jackie and Tobruk (Loladze *et al.*, 2014). Furthermore, when the Chinese Spring-Imperial rye chromosome substitution lines were independently challenged with closely related pathotypes at the seedling stage, pathotype 134 E16 A+ (+*Yr17* + *Yr27*) was comparatively more virulent relative to 108 E141 A-, suggesting a marked difference in virulence/avirulence and also host specificity (Fig. 3). Pathotype 134 E16 A+ and its presumed mutant derivatives are also characterized for virulence to resistance gene *Yr9* that was originally derived from *Secale cereale* (rye). Similar examples for both groups of *P. striiformis* f. sp. *tritici* pathotypes can also be drawn from barley, where isolate-specific resistance responses (quantitative trait loci, QTLs) were identified implying the involvement of different resistance genes or distinct mechanisms to the two isolates (Haghdoust *et al.*, 2018).

Some plant species are unexpectedly nonhosts to particular pathogen species. Rice – a distant relative of cereal crops species including wheat, barley and oat – does not have its own adapted rust or mildew pathogens. Both sugarcane and bamboo are closely related and originate from the same tropical climatic regions as rice, yet they have their own adapted rust pathogen species. Ayliffe *et al.* (2011) examined the response of numerous rice accessions to multiple cereal rust pathogens including: *P. graminis* f. sp. *tritici, P. triticina, P. hordei* and *P. striiformis* f. sp. *tritici*. Haustorial development was observed within rice mesophyll cells, as well as marked variability in hyphal size and morphology on the same leaf blade, however sporulation was not observed on any accession (Fig. 1). Ayliffe *et al.* (2011) concluded that rice's immunity to cereal rust pathogens was due to callose deposition, production of reactive oxygen species, and occasionally, cell death. One hypothesis is that preformed barriers (such as silica or increased lignification) or the presence of highly effective receptors capable of recognizing rust-specific PAMPs may protect rice from rust. Rice was reported to have 10 times the number of non-arginine-aspartate (non-RD) receptor kinases of Arabidopsis, suggesting that it has somehow expanded its ability to recognize a greater array of PAMPs produced by different pathogens (Dardick & Ronald, 2006). The fact that cereal rust pathogens can produce haustoria in rice indicates that they can evade PTI in this species, forming both pre- and post-developmental structures, but they do not appear to have the essential effectors to program cellular processes supporting biotrophy to the extent that supports pustule formation and sporulation. The possibility also remains that extremely rare susceptibility to some rust pathogens may also occur in rice genotypes that have not yet been challenged with the appropriate rust pathogen. Given that rice is more closely related to bamboo and sugarcane, it may be worth assessing its response to rust pathogens of these two grass species in addition to those that attack cereals.

Genetic studies of 'near' nonhost resistance

Several studies of mainly wheat and barley have investigated the presence and genetic basis of resistance to different heterologous cereal rust pathogens. More than 50 yr ago, Sanghi & Baker (1972) studied the genetic basis of resistance in wheat to diverse stem rust pathogen cultures. These studies determined that some common wheats, although not fully susceptible, supported significant colonization and sporulation by the rye stem rust pathogen

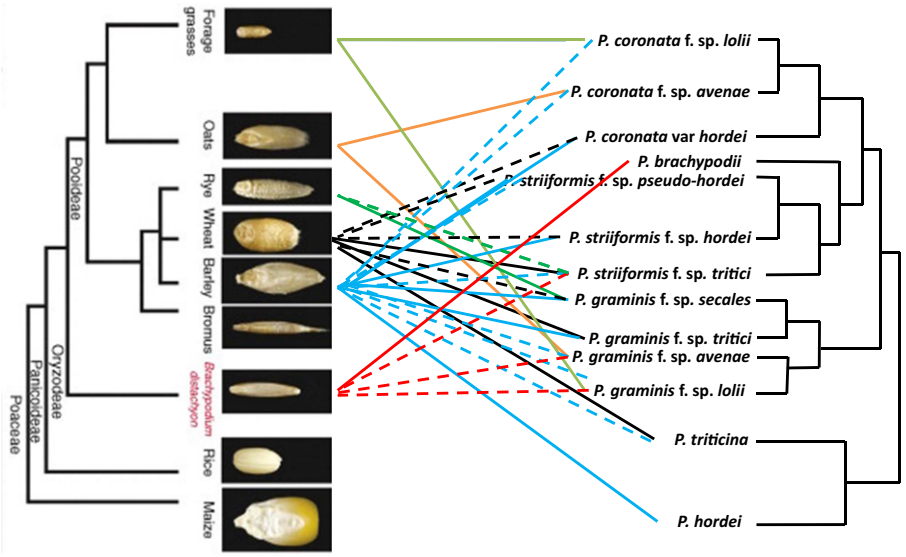

Fig. 2 Schematic outlining the host (solid lines) and near nonhost (dashed lines) specificity between different cereal and forage rust pathogens (*Puccinia* spp.) and the various host and model grass species in the Poaceae (Jin *et al.*, 1993; Atienza *et al.*, 2004; Figueroa *et al.*, 2013; Niks *et al.*, 2013; Dracatos *et al.*, 2016;). The phylogeny of the grass family was adapted from Opanowicz *et al.* (2008), with permission from Elsevier, and each of the receptive crop/forage species is represented by different line colours to differentiate the relative specificity to the different *Puccinia* spp.

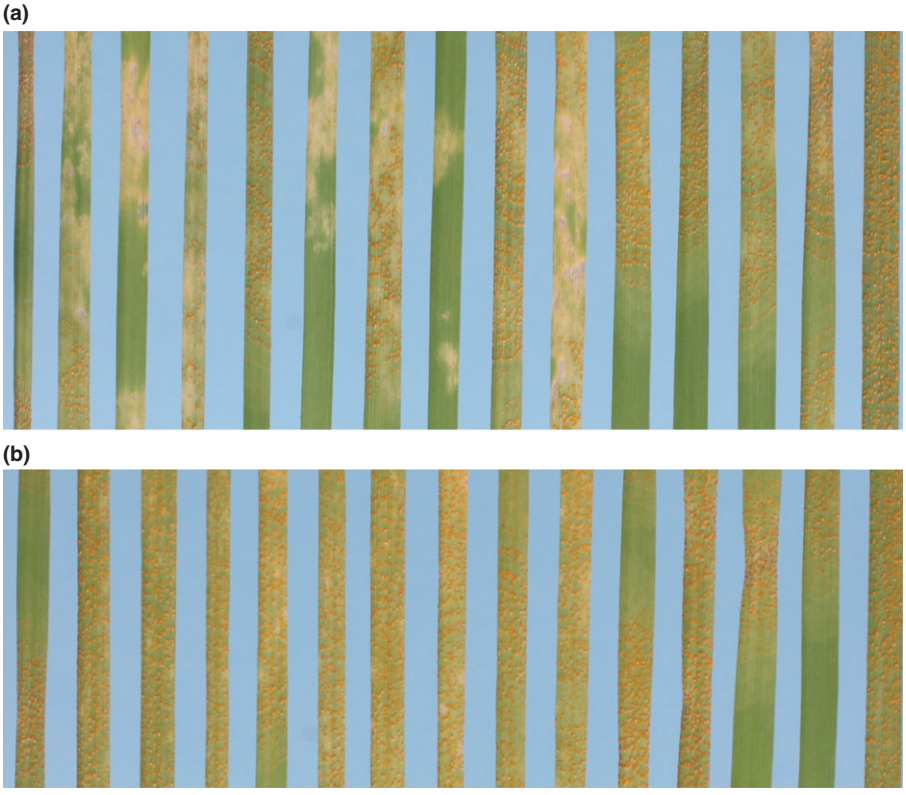

Fig. 3 Wheat seedling leaves (L to R) of Chinese Spring, 13 different rye (cv Imperial) chromosome substitution lines (including 1R, 1RL, 2R, 2RL, 3R, 3RS, 4R, 5R, 5RS, 6R, 6RL, 7R and 7RS) and the susceptible control Morocco infected with *Puccinia striiformis* f. sp. *tritici* pathotypes (a) 108 E141 A- and (b) 134 E16 A+ (+*Yr17* + *Yr27*) 16 d after inoculation. Pathotype 108 E141 A- differs from 104 E137 A- based on virulence for wheat stripe rust resistance gene *Yr6,* whereas 134 E16 A+ *(+Yr17* + *Yr27)* and 134 E16 A+ differ based on virulence for *Yr17* and *Yr27.*

(*P. graminis* f. sp. *secalis* – *Pgs*) and other *P. graminis* cultures with unusual avirulence genes. An early study determined that *Pgs* was also able to infect wheats carrying stem rust resistance gene *Sr11*. Selection within segregating populations led to the development of experimental wheat line W2691 that was somewhat susceptible to Australian isolates of *Pgs* (Luig & Watson, 1976). This finding established a new platform that enabled further inheritance studies of near-NHR in wheat, which determined that the stem rust resistance gene *Sr18*, present in most wheats, conferred resistance to *Pgs* but not *Pgt* (Baker *et al.*, 1970). This suggests that *Sr18* and a range of similar genes protect most wheats from *Pgs*, and in this way, play a role in host specialization (Baker *et al.*, 1970; McIntosh *et al.*, 1995). Unfortunately, inheritance studies to determine the involvement of genes like *Sr18* in protecting wheat from more distantly related *formae speciales* of *P. graminis* (e.g. *lolii*, *avenae*) are challenging due to a lack of wheat genotypes susceptible to these *formae speciales.*

In some grass species such as barley, a few accessions have been found to be moderately susceptible to heterologous pathogens suggesting peripheral host or near nonhost status

(Martens *et al.*, 1977; Atienza *et al.*, 2004; Jafary *et al.*, 2008). Previous research identified barley as an intermediate or near nonhost to most heterologous *Puccinia* species due to higher relative frequencies of susceptibility, mainly observed in accessions of diverse origin (Atienza *et al.*, 2004; Niks *et al.*, 2013). Martens *et al.* (1983) proposed that barley may indeed be the ancestral host for *P. graminis* based on the widespread susceptibility (yet never full compatibility) of barley to all variants of *P. graminis*. Atienza *et al.* (2004) used a similar approach to Baker *et al.* (1970) in developing an experimental barley line with susceptibility to *P. triticina* ('SusPtrit'). F_2 lines derived from crosses between four unrelated barley accessions of diverse origin, that were somewhat susceptible at the seedling stage to the heterologous wheat leaf rust pathogen (*P. triticina*), were identified and intercrossed, and lines with complete susceptibility selected in later generations. At seedling growth stages, SusPtrit was not only as susceptible to *P. triticina* as a regular wheat seedling, but also was susceptible to several other heterologous rust pathogen species including oat and *Lolium* grass-adapted *formae speciales* of *P. graminis* including *avenae* (Dracatos *et al.*, 2016) and *lolii* (Atienza *et al.*, 2004). Martens *et al.* (1977) identified a barley selection that was moderately susceptible to *P. graminis* f. sp. *avenae* (*Pga*), and further determined that a single gene was responsible for resistance to four oat-adapted *P. graminis* isolates, and showed that this resistance was distinct from the gene conferring resistance to *P. graminis* f. sp. *tritici* (*Pgt*). This implies that *Pga* carries avirulence effectors for resistance genes in barley that are absent or ineffective in *Pgt*. A further study by Dracatos *et al.* (2015) conversely found evidence that the barley stem rust resistance gene *Rpg5*, known to confer resistance to *Pgt* and *Pgs*, also conferred resistance to *Pga*, implying the presence of at least one similar recognized effector in all three *formae speciales*.

The ability to identify all contributing alleles for the immunity commonly observed for NHR may depend on population size, inoculation method and disease assessment. In barley especially, the immunity to numerous heterologous rust pathogens observed in cultivars such as Vada, Cebada Capa and Golden Promise was largely due to different sets of cultivar-specific QTLs with varying effects with the rare involvement of a common *R* gene (Jafary *et al.*, 2006, 2008; Yeo *et al.*, 2014; Niks *et al.*, 2015). Most of the studies using the barley–*Puccinia/Blumeria* model system that report on NHR being inherited quantitatively used a settling tower for inoculation. The settling tower method can be used to compare the infection frequency and the number of visible infection sites (VIS) per unit area relative to a susceptible standard genotype, and therefore enables the detection of quantitative variation in pustule density and latency period. Other methods (e.g. the Stakeman '0'–'4' infection type scale) that do not permit quantification of inoculum per unit leaf area are limited to the detection of genes of large phenotypic effect and are therefore more prone to bias for the detection of major gene resistance. The development of barley mapping populations and near-isogenic lines (NILs) based on the susceptibility of research line SusPtrit to both adapted and heterologous rust pathogens has permitted the identification of pleiotropic resistance QTL with overlapping specificities.

For instance *Rphq3*, a QTL identified at the seedling stage in Vada on chromosome 6H for partial resistance to *P. hordei* also conferred resistance to *P. graminis* f. sp. *tritici* and to heterologous rust pathogens *P. triticina* and *P. graminis* f. sp. *avenae* suggesting either very tightly linked QTLs or the same causal gene and mechanism of resistance (Qi *et al.*, 1998; Jafary *et al.*, 2006). The same locus also has been identified to function at the adult stage in the field to confer resistance to leaf rust (*P. hordei*) and was very prevalent across different international germplasm sources and highly interactive with partial/adult plant resistance gene *Rphq4/Rph20* (Ziems *et al.*, 2017). Numerous other examples have also been identified where genes in barley conferring partial resistance to leaf rust also are effective to heterologous rust pathogens.

Application, utilization and potential durability of NHR

Transferring resistance from one cereal species to another can be challenging using conventional approaches. Where possible, the system is dependent on interfertility between the host and nonhost species, and genetic studies are dependent on rare susceptibility in the near nonhost. Only two specific examples exist where QTL mapping has been possible by hybridizing interfertile host and nonhost species (Bothmer *et al.*, 1989). This is the ideal situation as it enables both trait dissection and the transfer of the NHR into the target plant species without the requirement for transgenic modification (Zhang *et al.*, 2009). The most relevant example of this was the stacking and transfer of four alleles from four recessive NHR QTLs from the nonhost *Lactuca saligna* to the host crop cultivated lettuce (*L. sativa*) using backcross inbred lines (Zhang *et al.*, 2009).

In wheat, many genes introgressed from other grass species have conferred all-stage resistance to all three rust pathogens. The origins of these genes from 'alien' species has not resulted in any greater durability, with most being overcome by matching virulence developing in the rust pathogen involved (Table 1). Likewise, different *P. graminis* cultures have been reported with unusual virulence/avirulence spectra, and experience has shown that rye-derived resistances are just as vulnerable to breakdown by wheat-adapted *Puccinia* taxa as those derived from *Triticum* spp. This suggests that the deployment of a specific *R* gene and its use in agriculture have determined probable durability, not where the *R* gene was originally sourced. Alternatively, if the NHR mechanism is under polygenic control (as in the *L. sativa* example) or similar in nature to the reported examples of the cloned pleiotropic adult plant resistance (APR) genes in wheat, then NHR in those instances may prove more durable (Wolday *et al.*, 2017). To date, two of the three pleiotropic partial APR genes (*Lr34Yr18/Sr57/Pm38, Lr46/Yr29/Sr58/Pm39* and *Lr67/Yr46/Sr55/Pm46*) in bread wheat have been cloned (Krattinger *et al.*, 2009; Moore *et al.*, 2015). Recent studies have reported on the transfer of *Lr34/Yr18/Sr57/Pm38* into different crop species with various levels of success. *Lr34/Yr18/Sr57/Pm38* was most

Table 1 Examples of rust resistance genes introgressed into *Triticum aestivum* from other grass species

Gene	Chromosomal location[A]	Origin	Pathogen	Presence of virulence
Lr19	7DL	*Thinopyrum ponticum*	*Puccinia triticina*	Yes
Lr24	3DL	*Thinopyrum ponticum*	*P. triticina*	Yes
Lr26	1BL	*Secale cereale*	*P. triticina*	Yes
Lr28	4AL	*Aegilops speltoides*	*P. triticina*	Yes
Lr37	2AS	*Triticum ventricosum*	*P. triticina*	Yes
Yr8	2D	*Triticum comosa*	*Puccinia striiformis* f. sp. *tritici*	Yes
Yr9	1BL	*Secale cereale*	*P. striiformis* f. sp. *tritici*	Yes
Yr17	2AS	*Triticum ventricosum*	*P. striiformis* f. sp. *tritici*	Yes
Sr22	7AL	*Triticum monococcum*	*Puccinia graminis* f. sp. *tritici*	No
Sr24	3DL	*Thinopyrum ponticum*	*P. graminis* f. sp. *tritici*	Yes
Sr26	6AL	*Thinopyrum ponticum*	*P. graminis* f. sp. *tritici*	No
Sr31	1BL	*Secale cereale*	*P. graminis* f. sp. *tritici*	Yes
Sr36	2BS	*Triticum timopheevi*	*P. graminis* f. sp. *tritici*	Yes
Sr38	2AS	*Triticum ventricosum*	*P. graminis* f. sp. *tritici*	Yes

[A]Chromosome locations for each gene designation were based on those cited in McIntosh *et al.* (1995).

recently transformed into durum wheat and unlike nontransgenic hexaploid and durum control lines, these transgenic plants showed robust seedling resistance to pathogens causing wheat leaf rust, stripe rust and powdery mildew (Rinaldo *et al.*, 2017). When transferred to barley, however, *Lr34Yr18/Sr57/Pm38* negatively impacted plant development (Risk *et al.*, 2013). It remains to be seen whether or not these alleles can be edited to ameliorate the deleterious effects on plant growth while retaining their ability to curb pathogen growth.

There is widespread evidence to suggest that NHR has been successfully engineered to control host-adapted pathogens, mainly in horticultural crop species such as strawberry (Silva *et al.*, 2015), peach (Jiwan *et al.*, 2013) and tomato (Scott *et al.*, 2010). The cloning of NHR genes from cereal crops will enable biotechnologists to determine the effectiveness of transferring such resistance as cassettes of multiple genes into closely related crop species. The *Rpg5* locus in barley represents an example of how a gene that confers resistance to multiple *formae speciales* of *P. graminis* could be transferred to multiple crops such as oat and even to forage grasses to protect those crops from stem rust. Although genomic advancements have been made for the transgenic improvement of forage species such as ryegrasses, the methodology is not quite as advanced for oat. The adoption of such technology and its impact on food security will depend upon public acceptance of cis-genic approaches to crop improvement. Such an approach, however, must be taken with extreme caution to avoid the risk of a possible host jump of *Pgt* to oat. Furthermore, as *Rpg5* is one of the only genes protecting barley from the highly publicized *Ug99* complex, if *Rpg5* were to break down, barley production also could be severely threatened (Steffenson *et al.*, 2009). The transfer of multiple genetic units with diverse mechanisms in resistance stacks is a more advisable approach for gene stewardship.

Recent gene editing technology using clustered, regularly interspaced, short palindromic repeats (CRISPR)-cas9 has been used broadly for gene knockouts, multiplex editing, interrogating gene function, and transcription modulation in both animals and plants (Khatodia *et al.*, 2016). One advantage of crop improvement using this system is that no transgene is introduced into the genome. CRISPR-cas9 is likely to be very useful as more genes are cloned, because existing susceptibility alleles from different cereal crops can be edited to function as resistance alleles creating new specificities. Alternatively CRISPR-cas9 would be extremely effective in editing/creating new loss-of-function recessive resistance alleles in diploid crop species such as barley and diploid *Triticum* spp. Such recessive genes often yield novel resistance mechanisms following molecular characterization that could be more difficult for breakdown by the pathogen. However, such technology also can be applied in polyploid crops. For instance, in a recent study the three homeologous copies (A, B and D genomes) of the wheat orthologue of the durable recessive barley 'mildew resistance locus' (*mlo*) were edited using CRISPR to produce wheat that was resistant to mildew (Wang *et al.*, 2014).

In conclusion, NHR is by far the most common form of resistance that plants have to protect against the diverse myriad of pathogens present in nature. NHR to heterologous pathogens can be expressed either during PTI or ETI, depending largely on the presence of various pathogen determinants or effectors/avirulence genes in the particular pathogen isolate. Recent studies on the host status of various heterologous rust and mildew pathogens in barley have determined that it is a marginal or near nonhost due to the presence of rare susceptibility. Therefore, barley has been used extensively in recent genetic studies as the preferred cereal model to identify resistance loci effective to heterologous pathogens, thus increasing the toolbox of available disease resistance genes for crop protection. In a majority of cases, the immunity or resistance identified to heterologous rust and mildew pathogens was quantitatively inherited with the rare involvement of major *R* genes. Whilst only a few studies have reported on the successful transfer of NHR between cereal crops, numerous examples exist in horticultural systems. Experience in wheat suggests that for more closely related species, the deployment of single nonhost genes

mediated by ETI from alien species may not be sufficient for durability. Due to the barriers associated with genetically engineered crops, the targeted modification of genes using CRISPR may provide an effective alternative for the transfer of NHR, especially as more causal genes are isolated and mechanisms are unravelled.

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