CURRENT REVIEW

A Time for More Booms and Fewer Busts? Unraveling Cereal–Rust Interactions

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Recent advances in our understanding of the nature of resistance genes and rust fungus genomics are providing some insight into the basis of resistance and susceptibility to rust diseases in our cereal crops. Characterized rust resistance genes, for the most part, resemble other resistance genes that interact with effectors intracellularly, but some have unique features. Characterization of rust effectors is just beginning but genomic information and technical advances in rust functional genomics will accelerate their characterization. The ephemeral nature of resistance in past varieties has made the design of cultivars with durable resistance a major focus for geneticists and cereal breeders. This includes strategies for deploying race-specific resistance genes that prolong their effects and methods of predicting which will be difficult for the pathogen to defeat. Identification of resistance genes with race-nonspecific effects is another strategy where recent breakthroughs have been made. Routinely combining the numerous genes required for complex resistance, whether specific or nonspecific, in elite cultivars remains a primary constraint to realizing durable resistance in most programs.

The cereal rusts, caused by various *Puccinia* spp., are among the most agriculturally damaging groups of diseases historically and in modern times. *Puccinia* rusts attack most of our major cereal species, including wheat, maize, barley, and sorghum. Their ability to produce multiple cycles of asexual spores (urediniospores) in a single season, the vast numbers of spores produced, and their ability to travel long distances create a potential for destructive epidemics if the cereal varieties are susceptible and weather conditions favorable. With few effective cultural control methods, production of the large acreages of cereals needed to feed the world has required the development of resistant crop varieties along with fungicide applications if possible and affordable. Therefore, rust resistance is a major objective of most cereal variety breeding programs around the world. Breeding for resistance has, for the most part, been an effective way of controlling the disease and the efforts spent on breeding, rust monitoring, and other research has shown a good return on the investment globally (Pardey et al. 2013). Although resistance breeding has been mostly cost effective, it is costly, and regional failures are not uncommon. The best-known failure in contemporary times is the recent

lack of wheat varieties showing resistance to stem rust (*Puccinia graminis* f. sp. *tritici*) in Eastern Africa due to the emergence of highly virulent races. This helped stimulate investments in applied and more basic rust resistance research, including the Borlaug Global Rust Initiative and federal funding for wheat breeding and rust genome sequencing. Although less acknowledged, the global failure of stripe rust resistance in wheat over the past decade has caused tremendous losses in East Africa and across Asia, North America, and Australia.

Efforts spent on incorporating rust resistance into varieties interfere with progress in improving other important traits such as yield and resistance to other pathogens and abiotic stressors, as well as various quality traits for different end uses. Unlike most traits, rust resistance is a moving target that sometimes moves very quickly. Because rust fungi evolve rapidly, a cereal variety that is highly resistant one year may be susceptible the next. Rust susceptibility is typically a fatal flaw for small grain varieties; therefore, otherwise excellent cultivars must be replaced, leaving both breeders and farmers disappointed. At the same time, resistance must be as cheap and effective as chemical control, because the low cost of fungicides and high grain prices experienced by many farmers around the globe in recent years have altered perspectives on the importance of variety resistance.

General features of characterized rust resistance genes.

Cereal species typically carry many genes for resistance to the rust species that attack them. In addition, species such as wheat, which can be hybridized with a large number of closely related species, have access to many rust resistance (*R*) genes. Over 150 wheat rust genes controlling resistance to leaf rust (*P. tritricina*), stem rust (*P. graminis*), or stripe (aka yellow) rust (*P. striiformis*) have been given the corresponding Lr, Sr, or Yr gene designations (Lagudah 2011; McIntosh et al. 1995). Although the actual genes have been identified for only a sample of these, the trend emerging is that most of them belong to the most typical class of *R* genes, the nucleotide-binding leucine-rich repeat (NB-LRR) genes. Examples include the wheat leaf rust *R* genes *Lr1* (Cloutier et al. 2007), *Lr10* (Feuillet et al. 2003), and *Lr21* (Huang et al. 2003); the stem rust *R* genes *Sr33* and *Sr35* (Periyannan et al. 2013; Saintenac et al. 2013); and the maize common rust *Rp1* and *Rp3* *R* genes (Collins et al. 1999; Webb et al. 2002). Two separate NB-LRR proteins are required for the *Lr10* resistance function (Loutre et al. 2009) while additional proteins required for the other resistances have not yet been identified but very likely exist. The proteins encoded by NB-LRR genes generally interact directly or indirectly with effector proteins in the host cytoplasm. A

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cytoplasmic location would be expected if most rust genes function to recognize the presence of effector proteins secreted from rust haustorial cells. Work with the flax rust system has indicated that this is generally the case. The L, M, P, and N loci of flax are composed of NB-LRR loci (Anderson et al. 1997; Dodds et al. 2001a and b; Lawrence et al. 1995). Avirulence (*Avr*) genes from the flax rust fungus (*Melampsora lini*) have been identified that are recognized by proteins encoded by genes at the L, M, and P loci. Each of the *Avr* genes were expressed in rust haustoria and predicted to encode proteins that were secreted from the haustoria. Expression of both the resistance proteins and the corresponding *Avr* proteins in plant cell cytoplasm caused a hypersensitive reaction (HR) (Catanzariti et al. 2006; Dodds et al. 2004). The nature of the *R* gene–*Avr* gene interactions has been best characterized for the L genes. L is a simple locus with an allelic series with different specificities. L proteins from the *L5*, *L6*, and *L7* alleles were shown to interact directly with a family of effectors coded by pathogen genes at the *AvrL567* locus. Individual *AvrL567* proteins caused HR of variable intensity with one or more of the three *L* alleles that interact with the family, in a pattern explaining the recognition specificities (Dodds et al. 2006). Interactions between the different *R* and corresponding *Avr* proteins varied in the strength in which they bound the effectors in yeast two-hybrid protein interaction assays, with only the stronger interactions corresponding to clear incompatible phenotypes in planta. Analysis of site-directed mutations and a series of chimeric L proteins indicated that the interactions involved multiple protein surfaces acting additively to determine the nature of the interaction with the effectors (Ravensdale et al. 2012). Sequence comparisons between the L alleles and the *AvrL567* genes indicated that both genes evolved under diversifying selection, as would be expected for strong selection for host and pathogen proteins changing to recognize or avoid recognition while they coevolved. Evidence for diversifying selection has also been observed for most of the other NB-LRR rust *R* genes where multiple alleles or family members have been compared. Whether they interact directly with fungal proteins or recognize their presence indirectly has generally not been determined.

The barley *Rpg1* gene is used extensively in the Upper Midwest states of the United States and prairie provinces of Canada for resistance to the wheat stem rust fungus (*P. graminis* f. sp. *tritici*). The resistance conferred by *Rpg1* is race specific but has provided resistance to a very broad spectrum of races. It has made a major contribution in protecting barley from wheat stem rust for most seasons over the last 60 years (Kleinohs et al. 2009; Steffenson 1992). *Rpg1* codes for a serine/threonine protein kinase (S/TPK), another common class of resistance protein and one that is known to participate in at least some NB-LRR-mediated resistances. Probably the most striking feature of the *Rpg1*-mediated interaction with *P. graminis* f. sp. *tritici* is the rapidity in which the interaction starts. The RPG1 protein is phosphorylated after inoculation with avirulent *P. graminis* f. sp. *tritici* isolates but not virulent isolates and is subsequently degraded. Phosphorylation can be observed within 5 min after inoculation of the leaf surface (Nirmala et al. 2010). The observation that arginine-glycine-aspartic acid tripeptide blocked infection and RPG1 phosphorylation prompted the purification of protein complexes by RGD affinity chromatography from germinated spores of the avirulent isolate (Nirmala et al. 2011). This led to the identification of two proteins: an 818-amino-acid RGD binding protein with a fibronectin type III and a breast cancer type 1 susceptibility protein domain and a 744-amino-acid protein with a vacuolar protein sorting-associated protein 9 domain and a coupling of ubiquitin to endoplasmic reticulum degradation domain. When infiltrated into *Rpg1* barley leaves together, the proteins elicit

RPG1 phosphorylation and an HR. Transcript analysis of the two proteins indicated that they were expressed in germinated and ungerminated urediniospores as well as purified haustoria. The two proteins interacted with each other and with functional RPG1 protein in yeast two-hybrid assays. The early events in the *Rpg1*-mediated resistance demonstrate that some plant rust interactions start well before haustoria formation. Histological analysis of rust resistance in some wheat cultivars has indicated that one component of their resistance is “pre-haustorial”, with quantitatively fewer infections advancing to the stage of haustoria formation (Wesp-Guterres et al. 2013). It is not known whether this prehaustorial resistance results from active responses to early recognition such as the *Rpg1*-mediated reactions or if it results from other physiological aspects of these cultivars.

Some of the other barley rust *R* genes have unusual but not completely unique features. The barley *Rpg5* gene provides resistance to some strains of the rye stem rust pathogen, *P. graminis* f. sp. *secalis*. The *Rpg5* protein codes for NB-LRR and S/TPK domains. It is unusual for both NB-LRR and S/TPK domains to occur in the same protein but this has also been observed in the protein encoded by the wheat *Tsn1* gene, which confers susceptibility to fungal pathogens expressing the protein toxin ToxA (Faris et al. 2010). The domains in the two proteins are arranged differently with respect to the N and C termini, however, and they probably arose through separate gene fusion events. The barley *rpg4* gene is unusual in the number of gene products known to be required and the fact that they are all very tightly linked genetically. The *rpg4* resistance is effective against many wheat stem rust races. Fine mapping and silencing analyses have indicated that *Rpg5*, an NB-LRR gene (*HvRga1*), and an actin depolymerizing factor (ADF) designated *HvAdf3* (Wang et al. 2013), all contribute to resistance. An ADF gene has also been found to be required for the RPS5/PBS1-mediated resistance of *Arabidopsis* to *Pseudomonas syringae* strains with *AvrPphB* (Tian et al. 2009). Genetic analysis indicated that there was a fourth unidentified linked gene required for the *rpg4* resistance which also mapped to the same genomic region. Another interesting feature of *rpg4* is that it segregates as though resistance is recessive, indicating that either two copies of one of the factors is required for noticeable levels of rust resistance or possibly that a gene in susceptible haplotypes (linked in trans) inhibits the resistance (Wang et al. 2013).

Two other wheat rust *R* genes have unique structures for disease *R* genes. The *Lr34* gene codes for an ABC transporter (Krattinger et al. 2009). *Lr34* provides partial resistance that is most apparent at the adult plant stage and is somewhat dependent on environmental conditions and genetic background (German and Kolmer 1992; Singh and Huerta-Espino 2003). Although its actual mechanism of action is not known, it provides resistance to multiple biotrophic pathogens, including stripe rust and powdery mildew (Krattinger et al. 2009; Lagudah 2011). The *Yr36* gene codes for a protein with an S/TPK domain as well as a domain with homology to steroidogenic acute regulatory protein-related lipid transfer (START) domains (Fu et al. 2009). Under high temperatures (25 to 35°C), *Yr36* confers resistance to all *Puccinia striiformis* isolates tested to date.

Realizing durable resistance.

The periodic losses due to the transient nature of rust resistance in cereal varieties has stimulated decades of discussion on why some cultivars remain resistant longer and how to select or identify this type of resistance. Factors that would affect how long the resistance in a cultivar remained effective would include the number of *R* genes that were independently con-

tributing to resistance as well as the resistance mechanism of the actual genes. Mechanistic factors include the nature of the pathogen components that interact with the resistance proteins or whether the *R* gene has true nonspecific effects, such as a gene that controlled physiological aspects of the plant independent of the pathogen, such as the *Lr34* gene (below). Good predictors of durability to date have been inconsistent and the only real measure has been the test of time. Certain durability trends have emerged, however. Cultivars with resistance that is controlled by several genes with small effects, typically mapped as quantitative trait loci (QTL), are often durable, as are those controlled by genes that function mainly in adult plants and not seedlings (Ayliffe et al. 2008; Lagudah 2011).

Clearly, genes with only small or adult plant effects should not be assumed to be race nonspecific. Some race-specific genes have relatively small effects on traits such as uredinia size. Spontaneous recombinants at the *Rp1* and *Rp3* loci have been identified that have the same specificity and, presumably, the same function as the progenitor gene but provide weaker resistance (Sun et al. 2001; Webb et al. 2002). Pathogen adaptation to resistance controlled by multiple genes with small race-specific effects might be slower because selection for mutations to virulence on any one gene would be weaker. Pathogen adaptation to wheat lines with such multigenic resistance has been documented (Johnson 1978), indicating that components of the resistance were at least partly race specific. Careful QTL analysis of partial *R* genes with multiple races have, in some cases, such as the barley leaf rust system (Qi et al. 1999), found both seedling and adult plant resistance QTL to be race specific. It is common for QTL with partial effects to not be detected consistently across environments, and very little is known about additive and epistatic interactions among rust resistance QTL (Singh et al. 2013; Yu et al. 2011). Environmental conditions, pathogen population structure, timing of epidemic development, and genetic background all seem to significantly influence the expression of partial resistance phenotypes. Thus, progress in characterizing genes underlying QTL has been slow because fine mapping is difficult and requires careful control of the environment and genetic background of the mapping family and detailed quantification of the resistance phenotypes. Race-specific adult plant resistance is very common in the wheat–stripe rust system (McIntosh et al. 1995; Parlevliet 1985; Sthapit et al. 2012) (X. Chen and E. Milus, *personal communication*), though publications are limited, presumably due to the low priority of mapping defeated race-specific *R* genes and perhaps a bias toward promoting durability of resistance phenotypes expressed in the adult stages. The wheat leaf and stem rust pathosystems have comparatively less evidence of race-specific adult plant resistance but examples such as *Lr12* demonstrate why care should be taken when characterizing new resistances (Singh and Bowden 2011).

Utilizing race-specific resistance.

Combining multiple effective *R* genes into a single cultivar is probably the most frequently strategized approach for durable rust resistance. The advantage of assembling a pyramid of effective *R* genes is diminished if the component *R* genes are deployed separately because it provides sequential selection of rust populations to overcome the components. Preventing individual deployment of these genes can be problematic, especially when the durably resistant cultivar is used as a parent for further breeding efforts. One approach is to recombine effective alleles of tightly linked *R* genes together into a single haplotype. This has been done in maize, where many of the common rust *R* genes map to a single complex locus designated *Rp1* (Hu et al. 1996). In other crops, such as wheat, the rust *R* genes are more distributed through the genome and useful combinations

of genes that are linked are more difficult to find. In addition, those that have been introduced from distantly related species sometimes recombine poorly in the introgressed region. Substantial investment has been made in reducing the size of alien translocations over the past several years. A few potential targets for linkage blocks have emerged over the past few years for wheat stem rust resistance, including genes newly introgressed from *Aegilops tauschii* that are reported to be in reasonably tight linkage with other genes effective against virulent East African races (Olson et al. 2013). Chromosome 2B of wheat harbors numerous stem rust *R* genes, including *Sr39*, *Sr40*, *Sr28*, *SrWeb*, *SrGabo56*, and *Sr47*, and active efforts are aimed at determining linkage relationships and assembling linkage blocks when feasible (Pumphrey 2012). The eventual success of such efforts will likely be determined by whether or not the resulting recombinant chromosomes have favorable alleles for other traits, including yield, quality, and resistance to other pests and diseases. Another pyramiding idea increasing in popularity is to clone and assemble numerous effective genes in cassettes to be used via transformation, where the genes are re-introduced as a single locus. This concept is being discussed for specific and nonspecific *R* genes or as combinations, because each strategy should benefit from accumulating several genes for durable or high-level resistance (Saintenac et al. 2013). This approach has the advantages of avoiding the linkage drag from multiple introduced chromosome segments and the resulting single locus is easier to manipulate in breeding. Cloning genes in wild Triticeae family species that have never been deployed in agriculture is being emphasized for *R* genes to be used in cassettes. Genes from other species will not necessarily be inherently more durable but their use only in combinations and not deployed individually may achieve this result. Designed cassettes may overcome the limitations of linkage to poor alleles of genes controlling other traits under selection, and would simplify stewardship and deployment.

Recognition of highly conserved pathogen-associated molecular patterns (PAMPs) has been generally found to be performed by membrane-spanning proteins with extracellular receptors rather than intracellular proteins such as NB-LRR (Dodds and Rathjen 2010; Jones and Dangl 2006). None of the rust *R* genes identified to date have properties of the characterized pattern recognition receptors (PRR) that detect PAMPs. This is probably because these more conserved functions are not typically variable in cereal species, not because PRR are not involved in rust resistance. If we borrow from what has been learned about how many phytopathogenic bacteria effectors function, it is likely that some rust effectors act to suppress responses elicited by PAMP recognition (Dodds and Rathjen 2010; Hogenhout et al. 2009). The function of the LysM effector of *Cladosporium fulvum* in suppressing chitin-mediated defenses supports this perception (de Jonge et al. 2010). The difference between what is considered a PAMP versus an effector is sometimes arbitrary (Thomma et al. 2011). Rust fungi may have PAMP-like molecules that are not yet apparent and effector-like proteins vary in their degree of conservation between the rusts. An example might be a molecule released during haustoria expansion and formation of the extrahaustorial matrix and could be recognized by an intracellular receptor. The *Yr36* gene is interesting in this regard because it codes for a protein with a START domain, which are often involved in lipid binding (Fu et al. 2009). *Yr36* has shown partial resistance to all stripe rust isolates tested under the appropriate environmental conditions but its durability is still largely untested in the field because it has not been widely deployed.

R genes that detect conserved effectors may provide more durable resistance, and the extent to which effectors are conserved between species can be easily tested for *Puccinia* spp.

because genome sequences of three are currently available. Probably more important than conservation is the function of the effector, because it would affect how easily the pathogen components recognized are lost or altered to escape recognition. Pathogen effectors in general are not equal in their contribution to a pathogen's fitness or pathogenicity, and this has been proposed as a method of predicting *R* gene durability (Ayliffe et al. 2008; Leach et al. 2001). Genomic analysis of rust pathogens have indicated that they carry large arsenals of effector-like proteins that are expressed during plant infection or specifically in haustoria and have motifs indicating that they are secreted (Duplessis 2011; Garnica 2013). Although the importance of any one effector likely depends on which others are present in a given isolates genome, methods to predict which ones represent essential processes in some strains may allow the prediction of *R* gene or gene combinations that are potentially more durable. With the recent demonstration that rust genes expressed in haustoria can be silenced, methods are now available to examine which genes are essential to pathogenicity (Panwar et al. 2013a and b; Yin et al. 2011). The silencing assays can be performed by introducing RNA fragments of the fungal genes into cereal seedlings using viral or *Agrobacterium* vectors and can be performed much more easily than making stable transgenic plants. Prediction of *R* gene durability requires that we know which *R* gene in the cereal cultivars or potential donor species corresponds to each effector. These associations are just beginning to be made but rust gene silencing assays and other techniques being developed for delivering effectors into cereal species will speed this process (Upadhyaya et al. 2014; Yin and Hulbert 2011). Understanding the cellular targets of important effectors may also inform predictions for durability once these targets are identified. Effectors that are recognized by *R* genes indirectly, through their modification of important cellular targets, may be more difficult to lose or alter to escape recognition because their function is more directly tied to their evolution. Modification of the host target has also

been proposed as a possible method to engineer rust resistance (Ayliffe et al. 2008) by interfering with the virulence function of the effectors.

Nonspecific resistance.

A few *R* genes are thought to confer resistance in a nonspecific manner, not through interactions with a specific effector. The best studied is the *Lr34* gene of wheat (syn. *Yr18/Sr57/Pm38/Sb1/Bdv1*). Thus, features of this gene have been considered when looking for other potentially durable genes. As mentioned above, *Lr34* confers partial resistance, mainly in adult plants, and provides partial resistance to multiple pathogens, including stripe rust, stem rust, powdery mildew, spot blotch, and *Barley yellow dwarf virus*. In fact, *Lr34* also confers resistance to barley leaf rust and powdery mildew in transgenic barley harboring a genomic copy of the wheat gene (Risk et al. 2013). The later feature is unusual because *R* genes are generally effective against only a single rust species. Another unusual but not unique phenotypic feature of *Lr34* is that it is associated with necrosis on the tips of adult leaves. Analysis of gene expression in the non-necrotic parts of these leaves found altered expression of multiple genes even in the absence of the pathogens (Hulbert et al. 2007). Many of the genes were those typically associated with abiotic stress responses, not typical defense response genes. In addition to *Lr34*, partial *R* genes *Lr67* (syn. *Yr46/Sr55/Pm46*) (Herrera-Foessel et al. 2011; Hiebert et al. 2010; Spielmeier et al. 2013) and *Lr46* (syn. *Yr29/Sr58/Pm39*) (Lillemo et al. 2013; Singh et al. 1998) confer resistance to multiple pathogens. Variable levels of necrosis on the tips of adult leaves are associated with each of these genes, even in the absence of pathogen challenge. Another multiple-pathogen partial rust resistance locus associated with a phenotype without rust infection is *Sr2* (syn. *Yr30/Lr27*) (Mago et al. 2011), which confers a variable level of purple-black pigment on glume, peduncle, and below-stem internode tissues (Kota et al. 2006). Numerous recent QTL mapping studies have begun to identify loci contributing to the “*Sr2*-complex” resistance that has remained durable for over 50 years (Singh et al. 2013; Yu et al. 2011, 2012).

The *Rp1* locus of maize is composed of a family of NB-LRR genes that confer race-specific resistance to common rust (*P. sorghi*) and some genes or gene combinations (haplotypes) show various phenotypic effects, including nonspecific resistance (Hu et al. 1996). The *rp1* genes mispair and recombine in meiosis, making recombinant genes and haplotypes that vary dramatically in gene numbers. Recombinant genes have been identified that respond to rust infection nonspecifically but also show spontaneous defense reactions, even without rust infection (Smith et al. 2010). Those with the strongest spontaneous HR destroy too much useful leaf area to be useful commercially. These extreme alleles, however, have been very useful tools for identifying naturally occurring allelic variants of genes controlling phenotypic variation in HR (Chaikam et al. 2011; Chintamanani et al. 2010; Olukolu et al. 2013) and identifying environmental conditions that affect *Rp1*-mediated responses. Other *Rp1* haplotypes have been identified that show partial resistance to common rust isolates that is unexpected from the individual genes they carry. For example, a recombinant haplotype carrying both *Rp1-D* and *Rp1-J* showed partial resistance to isolates that neither gene confer resistance to and also provided partial resistance to southern rust (*P. polysora*) (Hu et al. 1997). The resistance was not noticeable in seedlings but became apparent once the plants had six to seven leaves. *Rp1* haplotypes providing this nonspecific resistance were also associated with spontaneous defense reactions. These were mainly confined to the lower leaves of adult plants in most maize lines but showed more severe effects in certain genetic backgrounds.

Fig. 1. Phenotypes associated with nonspecific wheat rust resistance genes. **A**, Pigmented glumes on a wheat spike from the pseudo black chaff phenotype associated with *Sr2*. **B**, Leaf tip necrosis associated with the *Lr34* gene; flag leaf on left is from ‘Thatcher’ and the right is from an isolate carrying *Lr34*.

Potential drawbacks with nonspecific resistance.

A common feature of nonspecific resistance loci identified to date is the occurrence of pleiotropic phenotypes, even in the absence of pathogen challenge (Fig. 1). Near-isogenic lines with *Lr34* yielded approximately 5% less than *lr34* sibs in multiple field seasons in Mexico (Singh and Huerta-Espino 1997), presumably due to the physiological cost of reduced leaf area due to leaf tip necrosis in these genetic backgrounds and environments. Transformation of barley with *Lr34* from wheat resulted in severe leaf tip necrosis throughout the plant life cycle and reduced vigor and seed production (Risk et al. 2013). Although selecting for *Lr34* has undoubtedly been beneficial, it is not clear that the *lr34* “yield” gene should be ignored in all breeding programs or production areas in the future. The glume pigmentation associated with *Sr2* has also limited use of this gene historically, due to either negative effects on yield or breeder or farmer preference (Sheen et al. 1968; Singh et al. 2011). The CIMMYT Wheat Breeding program has continued to make impressive yield gains using combinations of *Lr34*, *Lr46*, *Sr2*, and other adult plant resistance loci extensively (Sharma et al. 2012; Singh et al. 2011) but it is unclear whether maintaining such resistance comes at a handicap. After 30 years of intensive breeding efforts at CIMMYT, other genes are undoubtedly being selected that limit such physiological effects; however, they are unknown and could limit application of the same resistance in other programs. Such modifiers and the “cost” of nonspecific resistance have been poorly studied, which will hopefully change in an era of perfect markers, inexpensive genotyping, increasingly employed genome-wide association studies, and integrated predictions of breeding value. From a practical breeding perspective, it will be a very long-term and challenging endeavor to increase the frequency of minor genes in entire breeding programs to the point that recovery of a high level of nonspecific resistance is routine in elite populations. At present, robust markers are only available for a limited number of nonspecific *R* genes, and the population size required to fix even three genes in many elite backgrounds is impractical for many breeding programs in the short term. The same limitations of managing adequate population sizes while stacking genes exist for race-specific gene pyramids.

Another common feature of nonspecific resistance is the more obvious influence of maturity and environment on resistance expression. Temperature, humidity, and light intensity are regularly credited with altering their expression. Poor expression of *Lr34* and *Lr46* under higher temperatures has been noted (Lagudah 2011; Zhang et al. 2008). Their poor effective-

ness in conditions when the environment is not appropriate has sometimes raised questions about whether they were effective against the prevailing rust races (Lagudah 2011). In contrast, *Yr36* and many of the adult plant resistances used in the Pacific Northwest of the United States have poor expression in seasons when spring temperatures stay cool, and are commonly referred to as high-temperature adult plant resistances (Carter et al. 2009; Chen 2013; Lin and Chen 2007, 2009; Qayoum and Line 1985; Santra et al. 2008). In fact, chemical control of stripe rust is recommended on cultivars with relatively high levels of adult plant resistance in seasons with severe epidemic potential (Sharma-Poudyal and Chen 2011) (X. Chen, *personal communication*). Considering the epidemiology of rust diseases, expression of resistance only in later stages of development provides a mechanism for oversummering or overwintering and for more rapid increases in inoculum during early stages of epidemic development. Stripe rust races presently distributed throughout wheat-growing regions of the world have apparently become more aggressive under both cooler and warmer conditions (Milus et al. 2009), which could further complicate the use of *R* genes with marked environmental sensitivity, especially in light of climate change predictions.

Future prospects.

Although both specific and nonspecific resistances have demonstrated their value in crop protection, neither has been employed optimally to date. Stewardship of effective *R* genes is more feasible moving forward than ever before and traditional *R* genes will have a role well into the future, as long as we continue to find or possibly create new specificities. Understanding effector function and distribution in pathogen populations and determining which effectors interact with *R* genes with histories of effective use will inform selection of *R* genes with more potential for durability. Characterization of genes with potentially nonspecific effects, including those mapped as QTL with relatively small but consistent effects, will reveal their function, which should shed light on how they can be combined for effective and durable resistance (Lowe et al. 2011). Additional work to determine their physiological costs, environmental dependencies, additive effects in combinations, and how easily rust fungi can adapt to their effects will help predict their value to breeding programs.

Transgenes are not currently used commercially to control diseases in cereals but this may change in the future. If a single transgene could control multiple rusts without a significant physiological cost, it could be a valuable tool to bolster breed-

Table 1. Overview of resistance in wheat to three rust diseases^a

Category	Leaf rust	Stripe rust	Stem rust
Resistance status	Few broadly effective <i>R</i> genes are available, and resistance in commercial varieties is largely based on combinations of regionally effective or recycled or previously defeated <i>R</i> genes and nonspecific resistance loci	Dozens of inadequately characterized <i>R</i> genes and adult plant resistance loci in commercial varieties. <i>Yr5</i> and <i>Yr15</i> are being used heavily around the globe in breeding programs. Race-specific adult plant resistance is common but poorly characterized	Most commercial varieties have adequate resistance based on <i>R</i> genes to local races but would be susceptible to races in the Ug99 lineage. <i>R</i> genes with broad effectiveness include <i>Sr22</i> , <i>Sr26</i> , <i>Sr32</i> , <i>Sr33</i> , <i>Sr39</i> , <i>Sr40</i> , <i>Sr47</i> , and <i>Sr50</i> ; many other genes confer resistance to Ug99-races, which could be combined with existing genes effective against local races
Potentially nonspecific resistance sources identified	<i>Lr34</i> (<i>Yr18/Sr57</i>), <i>Lr46</i> (<i>Yr29/Sr58</i>), <i>Lr67</i> (<i>Yr46/Sr55</i>), <i>Lr68</i> , and several inadequately characterized loci	<i>Lr34</i> , <i>Lr46</i> , <i>Yr30</i> (<i>Sr2</i>), <i>Lr67</i> , and numerous adult plant resistance loci, some of which have been well characterized, such as <i>Yr36</i> and <i>Yr48</i> , and many that have been inadequately characterized	<i>Sr2</i> , <i>Lr34</i> , <i>Lr46</i> , <i>Lr67</i> , <i>Sr56</i> , and numerous inadequately characterized loci
Relative global losses in recent years	Moderate	High	Low

^a *R* = resistance gene.

ing efforts. The observation that *Puccinia* genes can be silenced in haustorial cells by RNAi expression in wheat plants and that this can affect pathogenicity (Panwar et al. 2013a and b) has exciting implications. Genes that are conserved across *Puccinia* spp. and important to pathogenicity could be valuable targets for engineering resistance to multiple rust species. Simultaneously silencing several important genes could, in theory, provide durable resistance unless mutations that disarm rust fungus gene-silencing systems can occur without deleterious effects. Genes encoding protein effectors may not be the best targets for engineering durable resistance, especially when trying to control multiple rust species, because they typically appear to be less conserved between species (Duplessis et al. 2011). Because intracellular recognition of proteins is not necessary for this type of suppression to occur, genes involved in basic pathogenicity or more essential cellular processes in haustorial cells may be better targets. The genes examined by Panwar et al. (2013b) were sufficiently conserved to silence homologs in multiple rust species. Another possible target could be the *Iaam* genes that *Puccinia* spp. use to cause wheat to overproduce auxin in infected tissue (Yin et al. 2014). The gene is conserved across all three wheat rust fungi and transient silencing in *P. graminis* indicated that it was essential for full pathogenicity.

In the shorter term, development of cereal varieties with durable resistance is dependent on germplasm development. Transferring multiple genes, whether specific or nonspecific, to elite genetic backgrounds is an often overlooked and unfashionable necessity at the cornerstone of durable resistance. Straightforward germplasm development approaches exist but all are resource intensive, even if diagnostic markers are available with inexpensive genotyping, doubled haploid programs, and improved selection methods. Simply stated, germplasm development is perhaps the greatest limitation to realizing durable cereal rust resistance and responsible gene stewardship, even with tremendous existing and emerging resources (Table 1). One good race-specific gene in an elite cultivar is worth three in the bush to most plant breeders. If durable resistance is a desired outcome, not just a desirable idea, future efforts must increase emphasis on delivering regionally adapted germplasm that enables deployment of durable resistance.

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