Pyramiding for Resistance Durability: Theory and Practice

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ABSTRACT

Durable disease resistance is a key component of global food security, and combining resistance genes into “pyramids” is an important way to increase durability of resistance. The mechanisms by which pyramids impart durability are not well known. The traditional view of resistance pyramids considers the use of major resistance gene (R-gene) combinations deployed against pathogens that are primarily asexual. Interestingly, published examples of the successful use of pyramids in the traditional sense are rare. In contrast, most published descriptions of durable pyramids in practice are for cereal rusts, and tend to indicate an association between durability and cultivars combining major R-genes with incompletely expressed, adult plant resistance genes. Pyramids have been investigated experimentally for a diversity of pathogens, and many reduce disease levels below that of the single best gene. Resistance gene combinations have been identified through phenotypic reactions, molecular markers, and challenge against effector genes. As resistance genes do not express equally in all genetic backgrounds, however, a combination of genetic information and phenotypic analyses provide the ideal scenario for testing of putative pyramids. Not all resistance genes contribute equally to pyramids, and approaches have been suggested to identify the best genes and combinations of genes for inclusion. Combining multiple resistance genes into a single plant genotype quickly is a challenge that is being addressed through alternative breeding approaches, as well as through genomics tools such as resistance gene cassettes and gene editing. Experimental and modeling tests of pyramid durability are in their infancy, but have promise to help direct future studies of pyramids. Several areas for further work on resistance gene pyramids are suggested.

Durability of disease resistance is crucial to attaining global food security and agricultural sustainability (Brown 2015; Mundt 1994, 2014; REX Consortium 2016). Durable resistance is that which remains effective while being deployed widely over a long period of time and in an environment conducive to disease (Johnson 1984). This definition makes no assumptions regarding the genetics, mechanism of action, degree of specificity, or permanence of resistance (Johnson 1981). There are multiple approaches to increasing durability of resistance (Brown 2015; Burdon et al. 2016; Lof et al. 2017; Mundt 1994, 2014; Pink 2002), none of which should be considered inherently superior to the other, and all of which likely benefit from being combined (Mundt et al. 2002). The “best” approach may depend on practical concerns such as availability of resistance genes in a given breeding program (Mundt 1994), as well as the population genetics and population structure of the pathogen (McDonald and Linde 2002). Combining multiple resistance genes into a single

plant genotype (a genetic pyramid) is one of several successful approaches to increasing durability, and is the subject of this review.

Terminology associated with host plant resistance is often confusing, and sometimes controversial. In this review, I use the operational terms major gene resistance and minor gene resistance, recognizing that there are some genes that may not fall neatly into either category. Major genes will be considered those that often provide a high level of disease reduction, and tend to be race-specific and thus nondurable when deployed singly. Major genes are synonymous with R-genes, which function to recognize corresponding avirulence products (effectors) in the pathogen (Dangl et al. 2013; Jones and Dangl 2006). Minor genes are considered to be those that usually provide smaller disease reductions individually, combine additively to provide higher levels of resistance, and tend to be more durable than major gene resistance. In parts of the review, however, I will need to adopt the terminology used commonly by those working with cereal rust diseases. This terminology considers seedling resistance (also called all stage resistance) as resistance that is expressed at all host growth stages, and is equivalent to major gene resistance. By contrast, adult plant resistance (APR) functions only at the adult plant stage, and is usually incomplete in expression. APR thus might be considered a special case of minor gene resistance.

The traditional view of resistance pyramids considers the use of major resistance genes deployed against pathogens that are primarily asexual. Under this scenario, mutations at individual

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avirulence loci are assumed to be independent, and the probability of a pathogen overcoming the pyramid of resistance genes is postulated to be the multiplicative product of the frequencies of virulence corresponding to the individual resistance genes contained within the pyramid (Schafer and Roelfs 1985; Wheeler and Diachun 1983); this is sometimes called the “probabilities hypothesis” (Mundt 1990, 1991). Though perhaps a useful starting point for considering durability of resistance pyramids, there are several reasons why pyramids would not strictly follow the standard view (Mundt 1990, 1991). First, there are mechanisms other than, or in addition to, mutation number that may contribute to durability. These include the impacts of specific virulence combinations on pathogen fitness (Burdon 1993; Green and Campbell 1979; Vanderplank 1975; Wolfe 1973; Wolfe and Barrett 1977), the effects of multiple virulence mutations (Parlevliet 1981) on pathogen fitness, and synergism between major genes and minor genes expressed at the adult plant stage (see below). Though empirical data are inconsistent (Li et al. 1999; Pedersen and Leath 1988; Vanderplank 1978; Woo and Newcombe 2003; Young et al. 1994) residual effects of defeated resistance genes might sometimes contribute to disease reductions in pyramids. Second, multiple virulence may arise other than through an improbable series of independent mutations (Mundt 1990, 1991; Person et al. 1976), such as through deletion mutations (Burdon 1993; Flor 1960; Timmis et al. 1990), somatic recombination (Chen et al. 2017; Noguchi et al. 2006; Park and Wellings 2012; Zeigler et al. 1997), loss of avirulence genes that recognize multiple R-genes (Petit-Houdenot and Fudal 2017), suppressor genes that affect multiple avirulence loci (Gates and Loegering 1991; Jones 1988), and transposable elements (Faino et al. 2016; Hartmann et al. 2017; Howlett et al. 2017). Finally, since most major resistance genes allow for at least a small level of pathogen reproduction, it would be possible for the pathogen to accumulate virulence sequentially, rather than through simultaneous, independent mutations (Brown 2015).

There are two other issues often discussed relative to the use of resistance gene pyramids. First, one would expect pyramids to be most effective when resistance genes have not previously been used singly, though previous use of the single genes is probably the most common situation (Burdon et al. 2014; Pink 2002). Virulence even to nondeployed R-genes may escape detection owing to low sample size or lack of the corresponding R-gene in differential sets. In many

cases, the resistance genes constituting a pyramid are not known until years after release of a cultivar (Green and Campbell 1979; Kolmer et al. 1991). Further, it seems unlikely that breeding programs would be willing to forgo use of resistance genes singly when present in an agronomically favorable variety. Second, it has been suggested that pyramids will be less effective for sexual pathogens, which would be able to rapidly combine virulence against multiple resistance genes (McDonald and Linde 2002). There is little empirical information available to evaluate this logical suggestion, however.

Though resistance pyramids are usually discussed with regard to major genes, it is important to give some consideration to minor gene resistance in this review, for several reasons. First, despite decades of study, the degree to which major genes and minor genes differ in terms of biochemical/molecular mechanism is still not entirely clear and there is likely to be at least some overlap (Ellis et al. 2014; Kou and Wang 2012; Niks et al. 2015; Poland et al. 2008; St. Clair 2010). Increasing evidence for pathogen adaptation to minor gene resistance (Andrivon et al. 2007; Caffier et al. 2014; Delmas et al. 2016; Delmotte et al. 2014; Pariaud et al. 2009) further obscures the distinction between minor and major genes. Second, as will be discussed below, there are many important examples of durable resistance pyramids being associated with a combination of major and minor genes. Finally, pyramiding of multiple minor genes is usually required to attain an economical level of minor gene resistance. I will not review the underlying genetics of minor gene resistance, which has been covered elsewhere (Geiger and Heun 1989; Kover and Caicedo 2001; St. Clair 2010; Young 1996).

Below, I attempt to summarize more recent work on resistance gene pyramids and to propose directions for the future. Though there are some parallels among disease resistance pyramids, insect resistance, and pesticide resistance (REX Consortium 2016), I will discuss only genetic resistance to biotic pathogens in this review.

EFFECTS OF RESISTANCE GENE COMBINATIONS ON DISEASE LEVELS

Numerous studies have described the successful pyramiding of major genes for resistance to disease and other stresses (see examples in Joshi and Nayak 2010). Some of these studies have

TABLE 1
Examples of resistance gene pyramids that reduce disease levels below that of the most effective single gene

Host	Comparisons	Measurement	Reference
Barley scald	Backcrossed lines with 0, 1, or 2 genes	Disease severity in field	Brown et al. 1996
Bacterial blight of rice	Single R-genes versus pairs	Disease severity in field	Ahmed et al. 1997
Bacterial blight of rice	Pyramids of 2 to 4 R-genes	Lesion length in greenhouse	Huang et al. 1997
Wheat leaf rust	Lines with 1 or 2 R-genes or R-gene + APR gene	Reaction type in greenhouse and field; severity in field	Kloppers and Pretorius 1997
Bacterial spot of pepper	Near-isogenic lines with 1 to 3 defeated R-genes	Disease levels in greenhouse and field	Kousik and Ritchie 1999
Ascochyta blight of pea	Pyramid of two R-genes	Disease levels in greenhouse	Tar'an et al. 2003
Potato late blight	Stacking three R-genes using cassette transformation	Detached leaf assays and avirulence infiltration	Zhu et al. 2012
Bean rust	Lines with 1, 2, or 3 R-genes	Reaction type in greenhouse	Souza et al. 2014
Bacterial blight of rice	Two-gene versus three-gene pyramids	Lesion length in field	Pradhan et al. 2015
Rice sheath blight	Chitinase, SAR regulator, or both, on cassette	Detached leaf and whole plant assay	Karmakar et al. 2017
Wheat powdery mildew	Backcrossing of transgenic lines to attain allelic pairs of <i>Pm3</i>	Disease level in field	Koller et al. 2018

demonstrated that combining major resistance genes can reduce disease levels over that provided by individual ones (Table 1). There of course are also numerous examples of combining quantitative trait loci (QTL) to reduce disease (St. Clair 2010), which are expected to often function through additive reductions in disease levels. A synergistic reduction in disease levels provides an indication that resistance genes have been successfully combined and are being expressed, with the potential to contribute to durability. In the shorter term, such reductions may have little benefit to disease control if the individual, nondefeated genes have large impacts on disease. When individual genes provide insufficient disease control, however, such synergisms can be of significant immediate economic benefit. For example, two named genes have been used for the control of wheat eyespot, caused by *Oculimacula acuformis* and *O. yallundae* (Pasquariello et al. 2017; Sheng and Murray 2013). *Pch1* has resulted in substantial reductions of yield loss and fungicide use in the Pacific Northwest region of the United States. *Pch2* provides a lower level of disease reduction and has been deployed widely in Europe. Neither gene alone is sufficient in environments highly conducive to disease. However, we have recently shown that a combination of the two genes provide a positive synergism and substantially increased levels of disease control (Fig. 1). It also is important to note that this synergistic effect was discovered mostly by happenstance rather than design; not all resistance genes will interact positively (see Identifying and Combining Genes in Pyramids below).

EFFECT OF PYRAMIDS ON RESISTANCE DURABILITY IN PRACTICE

The preceding section provides no information about the potential durability of resistance gene pyramids. Indeed, evaluating durability is highly difficult, as it requires extensive deployment and evaluation over an extended period of time. Further, a description of the successful use of resistance gene pyramids, though crucially important, does not lend itself well to the replicated experiments most often published in refereed journals. Though I am certain that other positive case studies exist, the only clear examples with adequate description that I am aware of all involve rust diseases of wheat.

Wheat stem rust. The control of wheat stem rust through resistance gene pyramids is clearly one of the greatest successes in the history of plant pathology. Once the scourge of wheat production, there has not been a significant epidemic in North America since the mid-1950s (Kolmer et al. 1991; Singh et al. 2015). Potential mechanisms contributing to durability of these pyramids have been widely discussed and include the number of major genes in the pyramid (Green and Campbell 1979; Schafer and Roelfs 1985), resistance combinations that impart negative fitness effects on races carrying the corresponding virulence (Mundt 1990, 1991; Vanderplank 1975), and effects of combining the APR gene *Sr2* with major genes (Johnson 1981; Kolmer et al. 1991). *Sr2* contributes incomplete resistance to wheat stem rust at the adult plant stage, has been utilized in wheat cultivars for nearly 100 years, contributes to durability of major genes in pyramids, and has contributed to resistance durability throughout the world (Ellis et al. 2014). In North America, wheat varieties with varying “*Sr2* complexes” have been durably resistant to stem rust in North America when grown on millions of acres and over a timespan of decades (Roelfs 1988).

The International Maize and Wheat Improvement Center (CIMMYT) has an equally impressive record in controlling stem rust, but on the global scale. *Sr2* complexes have been a key component of stem rust resistance at CIMMYT (Rajaram et al. 1988; Singh et al. 2011; Singh et al. 2015), with pyramids of major genes being of lesser importance (Rajaram et al. 1988). Though *Sr2* has been used extensively for many years (Singh et al. 2015), other genes have subsequently been identified and utilized (Yu et al. 2014). Singh et al. (2015) noted that major genes *Sr22*, *Sr25*, *Sr26*, *Sr33*, *Sr35*, *Sr45*, and *Sr50* have potential to provide useful resistance against the newer Ug99 race group in Africa/Asia, provided that they are deployed in combinations. Thus far, however, Ug99-resistant varieties possess pyramids of four to five APR genes, and have provided very high levels of resistance (Singh et al. 2011). Studies of multiple biparental mapping populations segregating for APR showed *Sr2* to be present and to be the most effective APR gene against Ug99 in the field for each of those populations (Bhavani et al. 2011; Njau et al. 2013; Singh et al. 2013).

Australia provides an additional example of the successful use of resistance gene combinations for wheat stem rust control through its

FIGURE 1

Lodging caused by eyespot for wheat genotypes with differing number of resistance genes under severe disease pressure (top panel) and moderate disease pressure (bottom panel) in screening nurseries. From left to right, wheat genotypes are the cultivars Stephens, ORCF-102, Bobtail, Rosalyn, and the experimental lines OR12150047, OR12150048, OR12150051, and OR12150061. Each bar is the mean of three or four field plots (top panel) and four field plots (bottom panel) (C. C. Mundt, R. S. Zemetra, and A. F. Heesacker, unpublished data).

program of anticipatory breeding (McIntosh and Brown 1997; Watson 1981; Watson and Singh 1952). This program relied heavily on maintaining low rust population sizes through resistance, surveillance of rust races to estimate the danger of new virulence combinations, detailed knowledge of resistance genes present in breeding material, and breeding for resistance gene combinations to counter potential changes in virulence structure of the pathogen. At one point, the program even used mutagenesis to develop broadly virulent stem rust isolates for determining resistance genes present in breeding material. The program has utilized both single resistance genes and resistance gene combinations (Park 2007). Though there is evidence that *Sr2* may occur at lower frequency in Australian germplasm as compared with that of North America and the CIMMYT program (Mago et al. 2011), *Sr2* complexes have played an important role in Australia, in addition to that of other gene combinations (Ellis et al. 2014). Pyramids initially were constructed based on known phenotypic interactions of resistance genes singly and in combination, as well as the necrotic reaction associated with *Sr2* (McIntosh and Brown 1997). Later, molecular markers played an increasingly important role (Ellis et al. 2014).

Wheat leaf rust. The successful use of pyramids for durable resistance to leaf rust in spring wheat has recently been described in detail for Canada (McCallum et al. 2016) and more globally in earlier reviews (Rajaram et al. 1988; Kolmer 1996). Successes in controlling wheat leaf rust seem to parallel wheat stem rust in terms of APR playing an important role. In particular, the APR gene *Lr34* appears to be equivalent to *Sr2* for stem rust, in that it provides incomplete resistance in the adult plant stage, improves durability of other genes in combination, and has thus far been race nonspecific. *Lr34* encodes a putative ABC transporter protein (Krattinger et al. 2009), and also provides resistance against stem rust, stripe rust, and powdery mildew of wheat (Rinaldo et al. 2017), as well as to anthracnose and rust of sorghum (Schnippenkoetter et al. 2017), powdery mildew and rust of barley (Boni et al. 2018), and blast of rice (Krattinger et al. 2016) when transferred to those three cereal crops transgenically. In 2007 to 2009, cultivars containing *Lr34* occupied as much as 35% of the wheat area in the main wheat belt of Canada (McCallum et al. 2012). In Canada, the APR gene *Lr13* is no longer effective alone, yet still appears to improve durability of resistance in combination with other genes, and *Lr13* complexes have also played a dominant role in attaining durable resistance to leaf rust at CIMMYT (Rajaram et al. 1988). Some major seedling resistance genes have remained durable when combined with *Lr13* and/or *Lr34*, with *Lr16* providing very high levels of resistance in such combinations (Kolmer 1996). McCallum et al. (2016) noted the deliberate attempt to combine multiple resistance genes into a single cultivar (Pasqua) to attain durability to leaf rust. This cultivar maintained a high level of resistance to leaf rust from 1991 to 2014, despite the fact that three of the resistance genes (*Lr11*, *Lr13* and *Lr14b*) were defeated during that time period and two other genes (*Lr30* and *Lr34*) provide incomplete resistance when used alone. The APR gene *Lr68*, which is closely linked to *Lr14b* (Herrera-Foessel et al. 2012), might also have played a role in this durability, as *Lr68* was unknown at the time that Pasqua was developed and released.

Wheat stripe (yellow) rust. A lesser amount of information regarding pyramids is available for wheat stripe rust. Johnson (1981) mentioned development of four cultivars with combinations of race-specific major genes, with all four all becoming susceptible before or shortly after commercial release. However, the resistance genes utilized had a history on nondurability when used singly and the pyramids were not highly complex. Mallard et al. (2005) noted that the French wheat cultivar Camp Rémy maintained resistance since its release in 1980, and that specific combinations of seedling and APR genes resistance of this cultivar, as well as its resistant parent Cappelle Desprez, "... may constitute the key to their durable resistance against yellow rust." In the U.S. state of Oregon, the cultivar Stephens, released in 1978 (Kronstad et al. 1978), dominated

the wheat area of Oregon for over 20 years (Peterson 2001) and was also grown extensively in adjacent states (C. Mundt, unpublished data). Stephens maintained excellent stripe rust resistance during the time in which it dominated commercial wheat production (C. Mundt, unpublished data). It has been postulated that this durability was owing to a combination of major genes and APR (Chen 2005; Chen and Line 1995). More recently, however, the arrival of new aggressive, temperature-tolerant races of the stripe rust pathogen (Hovmøller et al. 2008) have resulted in a moderately susceptible field reaction for this cultivar due, at least in part, to earlier epidemic initiation (C. Mundt, unpublished data).

It is common for *Yr17*-virulent races that cause a susceptible reaction in the seedling stage to result in a moderately resistant reaction at the adult plant stage (Milus et al. 2015). Durability of wheat stripe rust resistance appears to often be associated with the combination of resistance gene *Yr17* with other resistance genes. Dedryver et al. (2009) noted that the *Yr17*-containing cultivar Renan had maintained durable resistance since 1989, despite the occurrence of stripe rust races virulent against that resistance gene. QTL analysis of a biparental mapping population identified three additional APR QTL contributing to the resistance of Renan, and some of the markers associated with *Yr17* were found to interact significantly with these QTL. Similarly, the cultivar Apache maintained durable resistance to stripe rust for 10 years, despite its wide cultivation in France (Paillard et al. 2012). QTL analysis showed that Apache likely contains *Yr17*, *Yr7*, and an APR QTL on chromosome 4B. Fang et al. (2011) noted that moderate resistance of the wheat cultivar Jagger had remained durable since 2000, despite Jagger or its offspring being grown on as much as 40% of the wheat areas of Kansas and Oklahoma at times. They found the resistance of Jagger to be controlled by a combination of *Yr17* plus a novel QTL on chromosome 5A. Though perhaps a bit early to designate as durable, stripe rust resistance in cultivar Bobtail winter wheat has maintained a high level of resistance to stripe rust in the Pacific Northwest (PNW) region of the United States since its release in 2012 (C. Mundt, unpublished data). The resistance of Bobtail, a selection from a mapping population, appears to be controlled by *Yr17* combined with a major gene from the parent 'Einstein' (Vazquez et al. 2015). Released in 1988, the cultivar Madsen, which contains *Yr17*, was the most commonly grown wheat cultivar in the state of Washington from 1994 to 2000 (Peterson 2001), and is still grown to some degree today (C. Mundt, unpublished data). Madsen has maintained moderate-to-high levels of resistance to stripe rust in the Pacific Northwest of the United States to this day, despite the introduction of highly virulent and aggressive races of stripe rust (C. Mundt, unpublished data) and a frequency of virulence against *Yr17* exceeding 80% (Wan et al. 2016). Preliminary QTL analyses for progeny of Madsen crossed with the susceptible cultivar Foote are consistent with the resistance of Madsen being controlled by a combination of *Yr17* with a novel QTL on chromosome 1AS (D. Vazquez, M. Vazquez, R. Zemetra, and C. Mundt, unpublished data).

The gene *Yr17* was transferred to hexaploid wheat from *Triticum ventricosum* via translocation of a large chromosomal segment rich in genes for resistance to a diversity of pathogens (Seah et al. 2001). As a result, one should be mindful of the potential for genes other than or in addition to *Yr17* being involved in the above studies.

Summary of durability in practice. Descriptions of resistance gene pyramids used in practice are relatively rare and restricted to a small number of similar pathosystems. Interestingly, examples of the successful use of pyramids in the traditional sense, i.e., combinations of major genes, are almost nonexistent. In contrast, most published descriptions of durable pyramids tend to indicate an association between durability and cultivars combining incompletely expressed, APR with other genes. This raises a series of significant questions: How durable are combinations of major genes? Is the association of APR genes with durable resistance a requirement, simply chance, or unique to the cereal rusts?

Interestingly, *Xa3* and *Xa4*, which are race-specific, adult plant genes for resistance to bacterial blight of rice, demonstrated durability when deployed extensively in Asia (Leach et al. 2001). This further supports the hypothesis of an association between APR and durability.

If including APR genes in pyramids is a key to durability, why might that be so? APR genes will result in less selection pressure on pathogen populations because resistance is incomplete and expressed during only part of the host's life cycle. It also is possible that APR genes and major genes are fundamentally different biochemically and instill different selection pressures against the pathogen. Indeed, some APR genes do not fall into the nucleotide binding leucine rich repeat (NB-LRR) class that is common for major genes, though it is probably safe to assume that others will (Ellis et al. 2014). In considering the historical effectiveness of *Sr2* against wheat stem rust, Ellis et al. (2014) noted that "... the fundamental question that has never been answered experimentally is whether the presence of *Sr2* has any direct effect on the durability of the associated stem rust R-genes in the complex. The major contribution of *Sr2* may be through the boosting effect on weak race specific R-genes that would otherwise be difficult to stack on the basis of their individual phenotypes in the absence of *Sr2*." Similarly, German and Kolmer (1992) suggested that the effectiveness of *Lr34* is partially due to its ability to increase the expression of other resistance genes in pyramids. It now appears that pleiotropic effects of APR rust genes to provide resistance to other diseases is not uncommon (summarized in Singh et al. 2015; Yu et al. 2014), which would seem to also distinguish APR from major genes. On the other hand, genes controlling multiple disease resistance also seem to be more common than previously recognized in general (Wiesner-Hanks and Nelson 2016).

CHOOSING DURABLE GENE COMBINATIONS

The preceding section strongly suggests that not all genes are equal in terms of their ability to contribute to the durability of resistance gene pyramids. The field experience of those highly familiar with a given pathosystem is thus invaluable to choosing effective resistance gene combinations (Mundt 2014; REX Consortium 2016). Others, however, have additionally asked whether there may be ways of choosing the most effective gene combinations in absence of prior information. For some populations of asexual pathogens, there can be a strong association between clonal lineage and specific sets of avirulence/virulence combinations (Midha et al. 2017; Nelson et al. 1994; Zeigler et al. 1995). This has led some to suggest that there may be genetic constraints associated with specific mutations to virulence within lineages. It thus has been hypothesized that durability of pyramids can be increased by combining resistance genes such that avirulence mutations occur at low frequency in each clonal lineage for one or more resistance genes, thereby excluding all pathogen lineages, i.e., the lineage exclusion hypothesis. Though such an approach may not provide permanence (Zeigler et al. 1997), it certainly would seem to have potential to increase durability as compared with random selection of resistance genes. Preliminary positive results have been obtained from attempted tests of the lineage exclusion approach for durable control of the rice blast disease (Gibbons et al. 2000; Gnanamanickam et al. 2000; Sivaraj et al. 2000; Tapiero-Ortiz 2001), though I am unaware of subsequent follow-up studies. Another potential approach to identifying effective resistance gene combinations is to consider the fitness impacts of the loss of specific effector genes in the pathogen. As avirulence genes also function as effectors of virulence and there is considerable redundancy among effectors (Cunnac et al. 2011; Jones and Dangl 2006), loss of different effectors may have very different impacts on fitness (Ponciano et al. 2003). Leach et al. (2001) noted several examples of correlation between fitness impacts of effector loss and durability of the corresponding resistance gene. They thus suggested that

pyramiding genes with corresponding to effector loss with high fitness impacts may be candidates for durability, a suggestion very worthy of further testing. Such evaluations will not be simple, of course, and results may be impacted by compensatory mutations (Parlevliet 1981; Wingaarden et al. 2005) and the potential for effector loss to influence both pathogen virulence as well as pathogen persistence in the environment (Vera Cruz et al. 2000).

Durability of pyramids may also be improved by utilizing genes other than traditional R-genes, which function via pathogen recognition and are easily overcome. Alternatives include "house-keeping" or pathogen-associated molecular pattern (PAMP) genes (Michelmore et al. 2013), which target pathogen genes that are highly conserved (Burdon et al. 2016; Dangl et al. 2013). Similar arguments might also be made for genes that control plant signaling responses to infection (Kachroo et al. 2017). Li et al. (2001) found that dominant, partially dominant, and recessive genes for resistance to bacterial blight of rice had different impacts on the pathogen population, and suggested that durability of pyramids may be increased by combining different types of genes. There has been much recent interest in selecting against, silencing, or editing plant susceptibility genes (Dangl et al. 2013; Hückelhoven et al. 2013; Pavan et al. 2010; Pessina et al. 2016; Sun et al. 2016; van Schie and Takken 2014), which are often recessive and may require pathogen mutation toward a positive function in order to be overcome.

IDENTIFYING AND COMBINING GENES IN PYRAMIDS

It has often been suggested that one effective gene in a pyramid will mask others, thus requiring the use of markers to ensure that the desired resistance genes have been combined (Burdon et al. 2014; Marais and Botes 2009; Pink 2002). Markers have contributed substantially to resistance breeding and construction of pyramids in recent decades, and ease of combining genes will improve with tighter markers and as we progress toward use of sequencing in place of markers. It also is important to note, however, that even with highly effective resistance genes, pyramids can sometimes be identified phenotypically. Few resistance genes give a true immune reaction and, in many cases, resistance gene combinations result in a lower phenotypic expression of a pyramid as compared with genotypes with fewer resistance genes. This is often the case for wheat rusts, for example, where infection types and disease severity can often identify successful combining of resistance genes (see Kloppe and Pretorius 1997 and references therein). Also, a simple and effective lesion length assay has been used for decades to evaluate resistance to bacterial blight of rice, and Pradhan et al. (2015) showed that this assay could consistently distinguish near-isogenic lines carrying two versus three resistance genes. Other examples are noted in Table 1.

Another issue is whether resistance markers are always indicative of the desired phenotype. Inconsistency could be due to markers that are insufficiently close to the resistance gene of interest in the population in which it was originally identified and/or in other genetic backgrounds. Marker technology is becoming increasingly more accurate and less expensive over time and, in the near future, many markers will likely be replaced by direct sequencing of resistance genes, thus eliminating the issue of marker distance. In addition, other methods may more directly predict resistance gene expression. For example, Zhu et al. (2012) combined three genes for resistance to late blight into a single vector and transformed it into a susceptible potato cultivar, and found that all 23 of the transformants expressed a resistance reaction against each of the three corresponding avirulence genes when agroinfiltrated into excised leaves.

Improved methodology for detection of resistance genes will not eliminate complexities associated with epistasis, however. In some cases, resistance gene combinations perform exactly as expected. For the late blight study mentioned above (Zhu et al. 2012), three R-genes derived from three different wild *Solanum* species were

expressed in all 23 *S. tuberosum* transformants. For bacterial blight of rice, two-gene ($n = 6$) and three gene ($n = 14$) backcrossed lines showed resistance relative to the susceptible parent, and all three-gene pyramids were more resistant than the two-gene pyramids (Pradhan et al. 2015). In contrast, Sakthivel et al. (2017) evaluated the same resistance genes (*xa5* + *xa13* + *Xa21*) studied by Pradhan et al. (2015), but found that expression of the *xa5* + *xa13* + *Xa21* combination differed substantially among five different host genetic backgrounds; complete resistance was expressed in only two of the five genetic backgrounds. Genetic interactions can also occur for increased disease resistance, of course (Li et al. 2001; Rouse et al. 2014; Vazquez et al. 2015).

There are multiple mechanisms by which epistasis for resistance may occur. In many cases, we merely know that some genes are not expressed in different backgrounds, with no determined mechanism. It is known, however, that suppressors may prevent expression of resistance genes, and have been proven or suggested to occur for the well-studied cereal rusts (Bai and Knott 1992; Hayes et al. 2006; Helguera et al. 2003; Knott 2000; Ma et al. 1995; Rouse et al. 2014; Stirnweis et al. 2014; Talajoor et al. 2015; Vazquez et al. 2015; Wu et al. 2015). There is every reason to expect that suppressors exist in other pathosystems as well. For example, Morel and Dangl (1999) identified nine gain-of-function mutations that led to the expression of the hypersensitive response to *Peronospora parasitica* on *Arabidopsis*. Epistatic interactions may be even more complicated in other cases. Kerber and Aung (1999) found that the *Lr34* APR gene in the cultivar Canthatch background inactivates the suppressor gene on wheat chromosome 7DL, allowing for expression of genes for resistance to wheat stem rust that are normally suppressed by the 7DL gene. Such studies suggest that “engineering” of biological systems is less predictable than that of mechanical ones, where the contribution of individual components and their combinations are much more predictable based on known mechanical properties. Thus, the ideal scenario for constructing resistance pyramids is to have dependable markers or sequence information to confirm presence of the targeted resistance genes, as well as a repeatable phenotypic assay to confirm expression of those genes.

BREEDING APPROACHES

Backcrossing is highly time-consuming process and “conservative” approach to breeding. Backcrossing to attain resistance pyramids may make sense when one is trying to improve resistance in a higher value crop with well-known and desired taste or other quality characteristic. For lower value field crops, however, breeders must often simultaneously make gains in yield, resistance, and other traits if they are to be competitive with other breeding programs. Procedures have been suggested for optimizing the backcross approach (Ishii and Yonezawa 2007a,b; Joshi and Nayak 2010; Peng et al. 2014). Further, alternatives to backcrossing may often be helpful. Simply choosing parents with multiple resistance genes will of course increase the probability of obtaining agronomically superior lines with more complex resistance package, which is likely an approach often used to attain pyramids in the past. More recently, modifications to back crossing have also been suggested. For example, Ruengphayak et al. (2015) described a pseudobackcrossing, marker-assisted method that allowed them to rapidly pyramid five major genes (including four for disease resistance) and three QTL from four different donors, while maintaining 75 to 81% of the genome of a rice cultivar with favorable agronomic characteristics. Similarly, Hickey et al. (2017) used four donors of resistance to four foliar diseases of barley in a modified backcross program utilizing “speed breeding” greenhouse growth conditions and multiple disease screens to produce 87 introgression lines in just two years. Subsequent field tests showed that all 87 lines incorporated multiple disease resistance, and the 12 most promising lines also showed favorable yield relative to the parents. Methods have also been discussed

to combine genes and increase durability of resistance for the case of hybrid cereal crops (Witcombe and Hash 2000).

Biotech approaches also have significant potential to combine resistance genes more rapidly than the traditional backcrossing approach. One such approach that has received considerable attention is the use of genetic cassettes that contain multiple resistance genes (Dangl et al. 2013; Ellis et al. 2014; Wulff and Moscou 2014). As mentioned above, Zhu et al. (2012) transformed a susceptible potato cultivar with three genes for resistance to late blight on a binary vector, using agroinfiltration. In a detached leaf assay, all 23 successful transformants were resistant to inoculated isolates of the pathogen, and showed a hypersensitive reaction to the three corresponding effectors. More recently, Karmakar et al. (2017) transformed into rice a chitinase overexpression gene, a regulator of systemic acquired resistance, or both genes. Transformants containing both genes showed a higher level of resistance to sheath blight than those carrying only a single defense-related gene. The CRISPR-Cas9 system (Belhaj et al. 2015; Doudna and Charpentier 2014) has resulted in a strong interest in using gene-editing approaches to construct resistance gene pyramids. Both gene editing and gene cassettes allow for development of cisgenic pyramids, which may be more societally acceptable than transgenic pyramids. Cassettes and gene editing also have potential to make substantial contributions to experimental studies of resistance gene pyramids. For example, field tests of resistance gene deployment strategies are greatly needed (Burdon et al. 2016; Mundt 2014), but development of gene combinations in similar genetic backgrounds can be a highly time-consuming process. Cassettes and gene editing could speed this process substantially. Similar arguments could be made for the development of pyramids to study the impacts of different gene combinations on pathogen fitness. New technologies seldom, if ever, provide the proverbial “silver bullet,” of course. Pyramids attained via use of cassettes will have the same difficulties as traditional breeding, such as the potential for suppression of resistance genes resistance/susceptibility tradeoffs between biotrophic and necrotrophic pathogens, may be technically difficult to transfer in some cases (Wulff and Moscou 2014), and it is unclear how pathogens will respond evolutionarily to the deployment of resistance gene cassettes. The CRISPR-Cas9 system, though very powerful, has its own set of technical challenges (Peng et al. 2016; Wolt et al. 2016; Zhao and Wolt 2017).

EXPERIMENTAL TESTS OF DURABILITY

Though durability of pyramids might be expected to be impossible to test empirically, a few such studies have been conducted. Two studies have asked whether residual effects of resistance genes can continue to provide disease control after the individual genes have been defeated. Kousik and Ritchie (1999) evaluated near-isogenic lines of peppers with defeated genes for resistance to bacterial spot of pepper in both greenhouse and field studies. Lines were available with 0, 1, 2, or 3 defeated genes. They found that the area under the disease progress curve (AUDPC) generally decreased with increasing number of defeated genes in the isolines, but concluded that these residual effects alone would not provide sufficient control of the disease. Ahmed et al. (1997) evaluated near-isogenic lines of rice with different major genes for resistance to bacterial blight. Using the AUDPC derived from field plots, they found that pyramids always had less disease than the best single gene for a pyramid of two defeated genes, as well as two different pyramids of a defeated with an undefeated gene. In addition, they found that the pyramids always provided greater disease control than the same genes used in mixtures. For both of these studies, however, one must consider the potential for compensatory mutations to eventually restore full virulence of the pathogen (Parlevliet 1981; Wijngaarden et al. 2005).

Two experimental systems have been used to test whether combining a major gene with minor gene resistance increases durability

of the major gene. In the first, the *pvr23* major gene for resistance to potato virus Y in pepper was introgressed into a susceptible background and into a background containing minor gene resistance with three identified QTL. The pathogen was passaged on the two pepper genotypes in sequential plantings in polyethylene tunnels. The major gene was defeated rapidly when in the susceptible host background, but not in the background with minor resistance (Palloix et al. 2009). In a subsequent study, four different QTL were identified that influence the rate of breakdown of *pvr23*, and three of these QTL were found to also contribute to quantitative resistance (Quenouille et al. 2014). In the second system, near-isogenic lines of oilseed rape were developed with and without the *Rlm6* major gene for resistance to Phoma stem canker. The lines were developed in a susceptible genetic background, and also in a genetic background with quantitative resistance. Residues of the four genotypes were carried over in different plots, and disease on the four host genotypes were tested in each of the four selection environments yearly. By year 5, disease on the lines for the treatment selected on the susceptible line carrying the major gene had increased substantially, but not on the line carrying both *Rlm6* and quantitative resistance (Brun et al. 2010). A follow-up study showed that virulence against *Rlm6* emerged in as few as three cycles of selection on the host genotype without quantitative resistance, but not until the eighth cycle of selection on the genotype with quantitative resistance (Delourme et al. 2014).

A 3-year field (plastic tunnel) study evaluated the impact of two major genes for resistance to root knot nematode of pepper (Djian-Caporalino et al. 2014). The study was conducted over a three-year period incorporating eight successive pepper crops. The pyramid greatly suppressed emergence of virulent races. Alternating the two different resistance genes between crops was the next most effective deployment strategy, and a mixture of the two genes was the least effective approach.

Roh et al. (2009) developed a sequential planting technique in the greenhouse to evaluate durability of resistance to rice blast. Though

used to quantify durability of specific rice cultivars, elite breeding lines, and near-isogenic lines carrying different R-genes, the same technique could be used to evaluate durability of resistance gene pyramids and to choose resistance genes that are most likely to be durable in pyramids.

MODELING DURABILITY

The difficulty of testing deployment strategies in the field makes mathematical modeling an obvious alternative. Three very recent studies have looked at similar questions at a large spatial scale. Lof and van der Werf (2017) developed a generic numerical model of multiple fields within a region. They considered four resistance genes and compared the durability of pyramids with that of sequential use of the same resistance genes, simultaneous use of the resistance genes, and a strategy utilizing both pyramids and single resistance genes simultaneously. Their general conclusion was that pyramids are the most durable approach only when virulence must be maintained at low levels. In all other cases, simultaneous use of the resistance genes in different fields was the most durable strategy. In a subsequent paper (Lof et al. 2017), they used integro-difference equations in a spatially explicit model in which the landscape of France was divided into 500 × 500 m grids and the model was parametrized for wheat stripe rust, including virulence frequencies that have been measured in the field and actual land cover data for wheat. They studied the same four deployment strategies as in their previous study (Lof and van der Werf 2017), but considered only two resistance genes. The model indicated that pyramiding was the most durable strategy only when virulence arises by mutation, and that simultaneously deploying single resistance genes with pyramids reduced durability of the pyramids. Finally, Djidjou-Demasse et al. (2017) used numerical approaches to compare resistance gene pyramids to that of deployment of the same genes among fields in a spatial mosaic for a model of plant virus diseases that included multiple resistance loci, seasonality, inoculum reservoirs, and a decadal time scale. They concluded that mosaics were more durable than pyramids for most of the parameter combinations evaluated.

Though models have much to contribute toward our understanding of resistance gene management, such studies are fairly limited in number and somewhat contradictory at this point in time. Obviously, some modeling outcomes would be expected to differ based on the biology of the specific pathosystem being studied. Modeling results can only be as good as the data used to parameterize the models, of course. There are some model inputs for which we have limited information, such as mutation rates and virulence costs. For other critical inputs, there are essentially no empirical data available. One example would be estimates of pathogen population sizes between cropping seasons. This is a crucial variable, as pathogen mutants with multiple virulence genes would be expected to initially occur at low frequencies and could repeatedly be lost to genetic drift if population sizes are small between seasons. Another example is the amount of pathogen migration that occurs among fields, which is difficult to measure empirically, but potentially crucial to predicting durability of resistance deployment (Djidjou-Demasse et al. 2017). In my opinion, the discipline of plant pathology has underinvested in the basic population biology studies that are necessary to develop reasonable estimates of such parameters, and the models ultimately need to be matched up with experimental and observational data from the field (Burdon et al. 2016; Mundt 2014).

CONCLUSIONS AND FUTURE DIRECTIONS

Resistance gene pyramids have been well-documented to provide durable disease control in several cases, especially for the cereal rusts. Though I expect that there are other successes in addition to those included in this review, published results on this topic are difficult to find. It is likely safe to assume that, on average,

BOX 1

TEN SUBJECT MATTER AREAS RELATED TO RESISTANCE GENE PYRAMIDS THAT ARE IN NEED OF FURTHER RESEARCH

- Mechanisms that contribute to pyramid durability
- Durability of pyramids with different classifications of resistance genes (major, minor, adult plant, resistance targeting pathogen housekeeping genes, lack of susceptibility genes, etc.). Given the history of pyramids in practice, the contribution of adult plant resistance to pyramid performance should receive special emphasis.
- Predicting the most durable resistance genes within a given classification
- Durability of pyramids for pathogens with differing life strategies (sexual, asexual, mixed mating systems, monocyclic versus polycyclic, fast versus slow rate of increase, etc.)
- Improved methods for identifying resistance genes in pyramids, including markers, sequencing, challenge with effectors, as well as traditional phenotypic scoring
- Epistatic interactions among genes in pyramids
- Breeding and biotech methods for constructing pyramids quickly and efficiently
- Population biology parameters relevant to predicting pathogen evolution toward pyramids
- Development of methods for experimental tests of pyramid durability
- Development of models to evaluate pyramid durability

resistance gene pyramids will be more durable than deployment of single genes sequentially. Unfortunately, little is known about the relative contributions of the various mechanisms that might contribute to the durability of resistance gene pyramids, and what resistance gene combinations may be most durable. At one level, a mechanistic understanding of durability may not matter, just as long as the pyramids function. However, what McIntosh (1988) said of wheat stem rust 30 years ago may still be true today, and for all plant diseases: “While wheats with low coefficients of infection in multilocal tests frequently possess multiple genes for resistance, the number of genes or type of gene interaction operating at each site has not been determined. However, information of this type will be essential if breeders are to assemble a diversity of such disease resistance as a means of achieving greater stability”. A better mechanistic understanding of pyramids is especially important for lower acreage crops, for which there are fewer breeders and pathologists available to provide empirical information regarding the relative durability of specific gene combinations. Genomics research is providing additional tools for understanding and deploying resistance gene combinations, and will likely play an increasing role over time. However, I agree with Vincelli (2016) that the keystone for resistance breeding is likely to remain with conventional approaches, at least for the foreseeable future. It is thus crucial that, in addition to genomics research, traditional field testing and race surveys are strongly supported.

Studies of resistance gene pyramids suggest a plethora of interesting topics that are of great importance to both the science of plant pathology and to improving food security through resistance durability (BOX 1). I hope that this review will stimulate pathologists and breeders to further investigate these topics.

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In the article “Pyramiding for Resistance Durability: Theory and Practice” by C. C. Mundt, names of two cultivars were transposed in the second sentence of the Figure 1 legend. The correct sentence is as follows: “From left to right, wheat genotypes are the cultivars Stephens, ORCF-102, Bobtail, Rosalyn, and the experimental lines OR12150047, OR12150048, OR12150051, and OR12150061.”