Plant Resistance and Strategies for Breeding Resistant Varieties

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Abstract

An explanation of the 'boom-bust' cycle of resistance breeding was provided by the gene-for-gene relationship between a pathogen and its host. Despite this understanding, most *R* genes continued to be deployed singly and resistance has been ephemeral. The reasons for breeding 'single *R* gene' varieties are discussed. Alternative strategies for the deployment of *R* genes and the use of quantitative race non-specific resistance have been advocated in order to obtain durable resistance. The feasibility of both of these approaches is discussed taking into account the impact of technologies such as plant transformation and marker-assisted selection. A change in focus from durability of the plant phenotype to that of the crop phenotype is advocated.

Keywords: *R* gene pyramids; multilines; mixtures; quantitative resistance; plant transformation; marker assisted selection; crop phenotype

The expectation following BIFFEN's (1905) demonstration that resistance in wheat to *P. striiformis* was controlled by a single locus, that production of resistant varieties would provide a permanent control of plant diseases proved to be unfounded when it was soon discovered that resistance was not always a durable phenotype. FLOR (1956) was the first to show there was a 'gene-for-gene' relationship between the pathogen's avirulence (*Avr*) genes and the resistance (*R*) genes of its host. Other have since shown that matching *R* and *Avr* gene pairs control the outcome of the host-pathogen interactions in many other systems (CRUTE 1985). The gene-for-gene theory provided an explanation of the 'boom-bust' cycle seen for many host/pathogen combinations. As a new resistant variety occupied an increasing area (boom), selection pressure against the matching avirulence in the pathogen population increased. If the resistance was based on a single R gene, a single mutation event at the corresponding *Avr* locus would result in a new virulent pathotype causing the resistance to 'bust'.

Despite this understanding, the most widely adopted 'resistance breeding' strategy throughout the 20th century remained the production of varieties with single effective *R* genes. Consequently breeders have been blamed for the perpetuation of the boom-bust cycle.

It is true that breeders continued to release many varieties with resistance based on single effective *R* genes. However, for many crops this was the only feasible means of releasing genes for resistance and for some diseases, notably virus diseases (HARRISON 2002) resistance determined by a single dominant gene has been durable.

Breeding of 'single gene' resistant varieties

There are many reasons why breeders continued to release varieties with resistance based upon a single effective *R* gene. The resistance was relatively easy to handle being qualitative with an easy to score phenotype and a high heritability and could generally be selected for at the seedling stage prior to breeders' field trials. Additionally marketing departments required a supply of new varieties. Even publicly funded breeders needed to produce new varieties/breeding lines to ensure continuation of funding. There was an apparent steady supply of *R* genes and breeding varieties with single effective *R* genes provided a stream of new

varieties. Commercial pressures dictated resistance needed to be incorporated into new varieties as quickly and efficiently as possible. Even though resistance was expected to 'break-down', being first to have resistant varieties for sale gave a market lead and a greater market share during the 'boom'. Introgressing a single dominant *R* gene is quicker and easier than attempting to introgress several *R* genes or breeding for polygenic resistance. In addition Plant Variety Rights (PVR) do not protect the genes released in a new variety. Other breeders are free to use the variety in their breeding programmes. A breeder therefore has no control over what others do with 'his' genes for resistance and there was little incentive to spend time and effort producing a variety with multigene resistance if others were going to split up the resistance gene combination.

Alternative forms of quantitative resistance were unacceptable in crops where quality was paramount since the level of control was insufficient. Effective *R* gene resistance provided complete disease control satisfying grower/retailer/consumer demands for 'clean' crops.

An unsustainable approach

Resistant varieties are often proposed as an integral part of the sustainable development of agriculture. Sustainable development has been defined as "...development that meets the needs of the present without compromising the ability of future generations to meet their needs" (The Brundtland Commission 1987). By this definition breeding for resistance has not been sustainable. Our ability to find new *R* genes is diminishing in many crops because the supply within the crop genepool is being exhausted. For example in wheat there are > 90 known genes determining race specific resistance to three rusts (*Puccinia striiformis, P. recondita* and *P. graminis*) and powdery mildew (*Erysiphe graminis*) nearly all of these are now ineffective (see CRUTE 1985). Many breeders are sourcing new R genes from the secondary genepool of wild relatives. However, these genes appear to be no different in terms of the likelihood of resistance 'breaking down' as *R* genes from crops. New *R* genes are a valuable genetic resource which need to be utilised in a more sustainable manner. Biotechnology will aid in the search for new resistance genes (MICHELMORE 1995) and there is the possibility of identifying or 'designing' new genes which may be more durable (CRUTE & PINK 1996). Cloned resistance genes can be deployed using transformation technology, however, unless deployed with care, the boom-bust cycle is likely to be perpetuated.

Alternative strategies for breeding resistant varieties

Gene Pyramiding. Pyramiding of *R* genes in a variety provides completely clean crops while the resistance is effective and is compatible with the need for crop uniformity. There are many examples of *R* gene pyramids. However, these have generally resulted from introgression of a new *R* gene into an adapted variety with an existing complement of 'defeated' *R* genes (PINK 2002). Effectively the main selection pressure in the pathogen population was against avirulence to the new *R* gene resulting in breakdown of resistance.

In theory pyramiding several 'undefeated' *R* genes should provide more durable resistance since mutational events at several *Avr* loci would be required to produce a new virulent pathotype. There has been debate whether larger gene pyramids are more durable (MUNDT 1990, 1991; KOLMER *et al*. 1991). However, there appears to be no correlation between numbers of genes for resistance and durability.

Marker assisted selection (MAS) for molecular markers linked to *R* genes or for the *R* genes themselves, enables pyramiding of several effective *R* genes (e.g. HUANG *et al*. 1997; HITTALMANI *et al*. 2000). Where *R* genes are clustered within the genome, this approach may be constrained by linkage limiting the gene combinations (LAW 1995). However, using MAS to select for rare recombinants containing resistance loci in coupling may create new *R* gene combinations for which the matching virulence may not readily evolve.

The prospect of cloning a series of *R* genes recognising the same pathogen, makes pyramiding by plant transformation feasible. However, in practice the time and effort needed to clone resistance genes means that this approach may only be likely in the foreseeable future for important pathogens of the most economically important crops.

The greatest danger in deploying *R* gene pyramids is that there is strong unidirectional selection pressure against the matching avirulence alleles in the pathogen population for evolution of a virulent pathotype. If the resistance is 'bust' the usefulness of all *R* genes in the pyramid is compromised. Strategies have been devised for deploying gene pyramids as mixtures in hybrid varieties using plant transformation (PINK & PUDDEPHAT 1999) or MAS (WITCOMBE & HASH 2000) to reduce unidirectional selection against the matching

avirulence alleles in the pathogen population. *R* gene pyramids are also vulnerable if any of the component genes are deployed singly in other varieties so that matching virulence to component *R* genes occurs within the pathogen population 'eroding' the effectiveness of the pyramid (PARLEVLIET 1997).

Mixing genes. Wild plants appear to use heterogeneity for *R* genes as a strategy for avoiding disease epidemics (e.g. BEVAN *et al.* 1993). Within-crop diversity is also a feature of subsistence agriculture in part to reduce damage by insects and diseases (SMITHSON & LENNÉ 1996). Growing crops heterogeneous for their *R* gene complement inflicts disruptive selection upon the pathogen population reducing the selection pressure against any one avirulence allele or combination of avirulence alleles and has been proposed as a strategy in developed agriculture for controlling crop diseases by the use of multilines (e.g. BROWNING & FREY 1969) or variety mixtures (e.g. WOLFE & BARRETT 1980). Multilines and mixtures can be distinguished by the relationship between their components. In multilines the components are usually closely related whereas the components of mixtures can be unrelated or distantly related. By implication variety mixtures are more haphazardly composed than multilines and are often considered as a deployment strategy rather than a breeding strategy. However, there is no reason why the components of mixtures should be any less carefully tested and selected than the components of multilines (SMITHSON & LENNÉ 1996). For the purposes of this paper mixtures and multilines are treated as examples of the same strategy to produce varieties heterogeneous for their *R* gene complement.

There is convincing evidence that crop heterogeneity for *R* gene complement reduces the incidence of disease in modern agricultural systems (WOLFE & FINCKH 1997). This appears to be due to a combination of several effects (GARRETT & MUNDT 1999) the relative importance of which can vary.

A major concern in using multilines/mixtures has been whether they would select for complex races. Theoretical (e.g. BARRETT 1978) and field observations (WOLFE & BARRETT 1980; CHIN & WOLFE 1984) on *E. graminis* in barley have suggested there is little unidirectional selection for complex races and their evolution will be slow. Any loss of resistance in the mixture/multiline is more likely to be due to an increase in frequency of a simpler pathotype with matching virulence to one component.

The contribution of multilines to modern agriculture has been relatively small; SMITHSON and LENNÉ (1996) cite only four examples of their significant use. The apparent advantage of multilines over mixtures is their greater agronomic uniformity which fits better with the perceived need for crop uniformity. However, they take time and effort to breed and it can be difficult to respond to changes in market requirements or in the frequency of virulence alleles in the pathogen population.

Mixtures were proposed as a pragmatic alternative to multilines and there are several examples (reviewed in SMITHSON & LENNÉ 1996; GARRET & MUNDT 1999; ZHU *et al.* 2000) which demonstrate their utility. It is possible to respond relatively rapidly to changes in the frequency of virulence alleles within a pathogen population or the relative importance of different pathogens (WOLFE 2000) by changing the mix of varieties. However, because breeders have continually introgressed new *R* genes into adapted varieties thereby maintaining defeated *R* genes in the crop, it can be difficult to find components with sufficient *R* gene diversity.

Their greater 'background' heterogeneity can buffer mixtures against abiotic stresses providing greater yield stability (SMITHSON & LENNÉ 1996). Problems of uniformity have, however, limited their acceptance (WOLFE *et al.* 1992). Breeding programmes specifically aimed at producing varieties as components for mixtures with similar agronomic traits but maintained heterogeneity for *R* genes might resolve this. An alternative would be the use of transformation to produce 'mix and match' multilines by transforming élite cultivars with different transgenes giving isogenic (uniform) components differing only in their *R* gene complement (PINK & PUDDEPHAT 1999). As with pyramiding transgenes, the transgenic multiline approach requires a collection of cloned *R* genes, which is only likely in the foreseeable future for the most important pathogens of major crops.

Non R gene resistance. There is a confusing plethora of terms used to describe 'non-*R* gene' resistance**.** However, in general the important aspects for a breeder that distinguish this resistance from *R* gene resistance are that it is quantitative and subject to environmental influences i.e. has a lower heritability. In addition because it is a 'rate reducing' resistance, resistant phenotypes can only be identified after several cycles of infection (i.e. seedling screens are generally ineffective). It therefore requires significantly more effort to breed for this type of resistance.

While there are examples of 'single gene' quantitative resistance generally this form of resistance is polygenic. Polygenes are now more commonly called quantitative trait loci (QTLs). QTL mapping allows the identification of associated molecular markers which

can be used in MAS to select for QTLs determining quantitative resistance making breeding for this type of resistance more efficient (LINDHOUT 2002). Breeding for polygenic resistance has been strongly advocated because the resistance is considered to be durable. However, there is evidence of isolate specific effects of QTLs for resistance (QI *et al.* 1999). This has consequences for any breeding strategy involving selection for QTLs.

The reliability of QTL mapping is limited by the accuracy of phenotypic assesment i.e. disease testing may need to be repeated in several environments to obtain robust data. It therefore may not be possible to identify all QTLs associated with a resistance. Thus it is unlikely that genotypic selection using markers linked to QTLs will entirely replace phenotypic selection. It may be better to combine MAS in early generations (e.g. F2) to ensure that all identified QTLs are retained in the breeding population with phenotypic selection in later generations. This would allow selection for the more resistant phenotypes possessing additional 'unidentified' QTLs of smaller effect. These QTLs while having a small effect in terms of the level of resistance may be important in determining the durability of resistance. While we still have little understanding of the basis of durability efforts should be made to maintain as many QTLs as possible in the breeding programme.

The future

It is difficult for breeders to decide on any one resistance breeding strategy. In the past decisions were taken based on information that enabled the breeder to focus on producing a resistant plant phenotype e.g. heritability, ease of selection etc. Often this has not resulted in durable resistance. An approach which shifts emphasis away from selection for a resistant plant phenotype to one where a resistant crop phenotype is the ultimate aim (PINK 2002). In terms of plant diseases this will be an economically acceptable level of disease. The decision can then be made as to the best breeding strategy to adopt to achieve this level of crop resistance. Host plant resistance would need to be integrated into a crop management system aimed at achieving a desired crop phenotype which included quality and yield traits. This will not only have to take into account the availability of plant resistance genes (*R* genes and/or QTLs) but also other factors effecting crop phenotype such as agronomy, breeding system, alternative control measures and most importantly the potential of the pathogen to evolve new pathotypes (MCDONALD & LINDE 2002). By integrating host plant resistance into a crop management system it is possible to achieve a durable crop resistance phenotype even though the resistance phenotype of individual plant genotypes is not durable (PINK 2002).

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