Pre-breeding for disease resistance in wheat – the stagonospora nodorum blotch example

Manisha Shankar, Michael Francki & Rob Loughman

Department of Agriculture and Food, 3 Baron-Hay Court, South Perth, WA 6151
Tel (08) 9368 3533
Fax (08) 9368 2958
Email mshankar@agric.wa.gov.au

'Pre-breeding' is an alternative term used for 'genetic enhancement', and in recent times it has become an essential, planned part of all plant breeding activities[1]. The main objectives of pre-breeding for disease resistance are to increase the efficiency of breeding by providing breeding programs with (i) molecular markers linked to effective disease resistance genes, (ii) improved germplasm and associated genetic knowledge that enhance resistance expression and diversity, (iii) improved parental stocks which can be readily utilised within breeding programs and (iv) improved selection methodologies. This article discusses the various activities involved in a pre-breeding program targeting disease resistance with recent outcomes of genetic enhancement for adult plant resistance to stagonospora nodorum blotch in wheat.

In Western Australia (WA) the main fungal leaf disease constraints in wheat production include stagonospora nodorum blotch (SNB) caused by *Stagonospora nodorum,* yellow spot caused by *Pyrenophora tritici repentis,* stem rust caused by *Puccinia graminis* f.sp. *tritici,* leaf rust caused by *P. triticina* and stripe rust caused by *P. striiformis* f.sp. *tritici.* Resistance is the preferred means of controlling these diseases because it is easy to use, cost-effective and reduces the need for other methods of control, including the application of chemicals. Genetic resistance within locally adapted germplasm is limited because of the lack of genetic diversity. Also, pathogens, particularly rusts, evolve over time and acquire virulences on previously effective resistance genes, further reducing the availability of effective disease-resistant genes. Pre-breeding for disease resistance is, therefore, essential not only to increase the efficiency of breeding for resistance but also to broaden the genetic base of resistance.

An example of successful pre-breeding for disease resistance in WA is that of adult plant resistance to SNB which is one of the most severe fungal diseases affecting wheat production in WA. The main activities in a pre-breeding program targeting disease resistance, exemplified here with SNB include:

(i) Identification of new resistances

The first step includes identification of sources of resistance within the existing germplasm and from various resources worldwide. Testing over multiple years, in various environments and at different growth stages can identify resistance as broadly effective. Potential resistant donors identified can then be directly incorporated into breeding programs or can be used for population development, identification of molecular markers and mapping of novel resistance genes.

Figure 1. Plots inoculated at heading with a spore suspension of *Stagonospora nodorum* and covered with moist plastic chambers for 48 hours.

Figure 2. Flag leaf necrosis caused by *Stagonospora nodorum* on a doubled haploid line of population 6HRWSN125/IGW2074 220°C degree days after inoculation.

Based on a worldwide survey of 3500 wheat lines we used sequential field testing (evaluating SNB leaf disease severity, plant maturity and height over a number of years) to identify elite resistant lines. Final selection of SNB-resistant donors included assessment of grain quality, avoiding soft grained lines with low flour yield and high α-amylase activity as these factors were likely to impact on future use in breeding. Through collaboration we also identified resistant material in different genetic backgrounds such as winter wheats. Some of these resistant donors were used for population development, identification of molecular markers and mapping of novel resistance genes while some others were directly incorporated into breeding programs.

(ii) Population development

For understanding inheritance of genes and identification of molecular markers linked to resistance we need mapping populations. Several different types of populations may be utilised for mapping. F2 populations are the simplest types of mapping populations with the main advantages that they are easy to construct and require only a short time to produce. However, they have the complication of having to deal with heterozygous individuals and the constraint that only a single assessment can be made per individual. These populations are suitable for mapping single major genes such as seedling genes for rust resistance in wheat. Recombinant inbred line (RIL) populations are produced by inbreeding F_2 individuals and consist of a series of homozygous lines, each containing a unique combination of chromosomal segments from the original parents. The time required to produce RIL populations is a major disadvantage, as it usually requires six to eight generations of inbreeding for these populations to be sufficiently homozygous. Doubled haploid (DH) populations are produced by regenerating plants by the induction of chromosome doubling from pollen grains; or by chromosomal doubling of haploid embryos produced by wide crossing wheat with maize. The major advantage of RIL and DH populations is that they produce homozygous or 'true-breeding' lines that can be multiplied and reproduced without genetic change occurring. These populations allow for replicated trials being conducted across different locations and years and are, therefore, ideal for mapping quantitatively inherited traits such as resistance to necrotrophic foliar diseases in wheat.

Our studies focused on three different resistant sources for SNB. A spring wheat resistance source 6HRWSN125 from CIMMYT, winter wheat sources P91193D1 and P92201D5 from Purdue University (USA) and a locally bred hard wheat source EGA Blanco. Based on the anticipated quantitative resistance to SNB, the following three mapping populations of around 250 individuals each were developed: (a) DH population 6HRWSN125/IGW2074 (b) DH population EGA Blanco/Millewa and (c) RIL population P92201D5/P91193D1.

(iii) Phenotyping

Phenotyping for disease resistance involves screening all individuals of a mapping population for disease expression. Although a qPCR protocol to measure fungal biomass, using the wheat-*S. nodorum* pathosystem has been established[2], visible disease response still serves as the primary means by which variation in resistance is investigated. Frequency distribution of individuals for resistance within a population indicates the type of inheritance. For example, in a DH or a RIL population a bimodal frequency distribution of 1:1 (resistant:susceptible) indicates single major gene inheritance, while a continuous distribution indicates quantitative inheritance or that the trait is conditioned by many genes or quantitative trait loci (QTL) with relatively small effects. In cases where resistance is associated with single genes, genetic effects are large in magnitude and detection is straightforward. In contrast, quantitative resistance is more difficult to investigate, since the effects of individual genes are small and phenotyping experiments must be performed with high levels of precision. It is important to conduct these experiments over multiple years and in different environments with adequate replications and controls so that a certain level of accuracy is achieved. Accurate phenotypic data which are reproducible are best able to identify the genetic variability within experiments.

We phenotyped the three SNB mapping populations for flag leaf resistance and glume resistance over multiple years and in different environments. The independent and complex genetic inheritance of SNB resistance in seedlings, flag leaf and glume coupled with confounding effects of maturity and height on

disease expression made the identification of true resistance a challenging task. Phenotyping methodologies were developed to assess true resistance by reducing effects of height and maturity on disease expression. This involved measuring the plant's response to a single cycle of infection by conidial spray inoculation of individual lines when glumes were fully emerged, followed by incubation in a plastic humidity chamber and disease evaluation by visual ratings at set thermal times (degree days). We observed continuous distributions in all three populations, indicating the quantitative inheritance of resistance to this disease.

(iv) Genotyping and QTL analysis

A parallel pre-breeding activity is genotyping individuals of a mapping population for DNA markers which denote randomly located genetic differences between the DNA of individuals. Generally, these markers do not represent the target genes themselves but act as signs or flags[3]. The first step in genotyping is to identify DNA markers that reveal differences between parents (that is, polymorphic markers). Polymorphic markers are then screened across individuals in the mapping population, with reference to the parents. A linkage map is then constructed, which involves coding data for each DNA marker on each individual of the population and conducting linkage analysis using computer programs. Linkage between markers is usually calculated using odds ratios (that is, the ratio of linkage versus no linkage). This ratio is more conveniently expressed as the logarithm of the ratio, and is called a logarithm of odds (LOD) value or LOD score[4]. LOD values of >3 are typically used to construct linkage maps.

In simple terms, QTL analysis is based on the principle of detecting an association between phenotype and the genotype of markers. Markers are used to partition the mapping population into different genotypic groups based on the presence or absence of a particular marker locus and to determine whether significant differences exist between groups with respect to the disease trait being measured[5,6]. A significant difference between phenotypic means of the groups indicates that the marker locus being used to partition the mapping population is linked to a QTL controlling the disease trait. Once a marker that is tightly linked to a particular resistance gene or QTL is identified, breeders may use this as a diagnostic tool to identify plants carrying that gene or QTL.

It is crucial that the processes of genotyping and phenotyping are as accurate as possible to produce reliable QTL maps. Genotyping errors can affect the order and distance between markers within linkage maps while a reliable QTL map can only be produced from reliable phenotypic data.

Genetic linkage maps were constructed for the three SNB mapping populations and QTL for *S. nodorum* flag leaf resistance on chromosome 1BS, 2AS, 2DL and 5BL and glume resistance on 4BL and 2DL were consistently detected in at least two environments and accounted for 10–40% of the total phenotypic variation in various spring and winter genotypes[7-9]. The deployment of various QTL for *S. nodorum* resistance in susceptible genetic backgrounds relevant to Western Australian environments has been undertaken and the effects of different QTL for improving resistance to SNB is currently being investigated through phenotypic evaluation.

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Biographies

Dr Manisha Shankar is a Senior Plant Pathologist at the Department of Agriculture and Food Western Australia (DAFWA) and an Adjunct Senior lecturer at the University of Western Australia. Her program focuses on enhancing resistance to foliar diseases of wheat through the development of genetic knowledge, genetic resources, breeding tools and selection methodologies.

Dr Michael Francki is Manager of Biotechnology at the Department of Agriculture and Food Western Australia and is Adjunct Professor at Murdoch University (Western Australia) and the University of Nebraska (Lincoln NE, USA). His program is focused on molecular genetic analysis of trait variation in wheat, identifying gene and QTL combinations for desirable phenotypes and delivering pre-breeding outcomes for adoption and utilisation by commercial wheat breeding companies.

Dr Robert Loughman is Director Genetic and Product Innovation, Grains Industry at the Department of Agriculture and Food, Western Australia. His personal research interests include genetic improvement for disease resistance in cereals.