Mutations in Wheat Leading to Enhanced Resistance to the Fungal Pathogen of Yellow Rust

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Abstract

The isolation and study of plant resistance genes is revealing a story more complicated than the gene-for-gene hypothesis originally implied. The story of resistance is complicated even further by the discovery of genes that appear to have a negative effect on resistance. Early studies in the wheat line Hobbit 'sib' identified a number of chromosomes that reduced the level of field resistance to the fungal pathogen *Puccinia striiformis* f.sp. *tritici*, the causal agent of yellow rust on wheat. From a series of deletion mutants generated in Hobbit 'sib' a number of mutant lines were selected that gave enhanced resistance to yellow rust. The phenotypic, genetic and molecular characterisation of some of these mutants is presented.

Keywords: mutants; wheat; yellow rust

INTRODUCTION

The isolation and study of plant resistance genes is revealing a story far more complexed than the gene-for-gene hypothesis originally implied. In addition to resistance triggered by single, seedling expressed, race-specific genes are resistances expressed only at later, adult plant growth stages. Although some cases of adult plant resistance (APR) have been shown to be single, race-specific genes many are polygenic, each individual gene conferring only a partial resistance.

In addition to resistance genes that inhibit pathogen invasion and disease establishment, there are genes that enhance the progress of the disease and have been termed susceptibility or suppressor genes. A number of examples of specific inhibition of seedling-expressed resistance are available in the literature. The introduction of yellow or brown rust resistance genes from diploid wheat accessions into a hexaploid background has often resulted in the loss of the resistance phenotype (KEMA *et al*. 1995; MA *et al*. 1995; ASSEFA & FEHRMANN 2000). Specific suppressors of stem rust resistance genes have been identified in the cultivar Canthatch on chromosome 7DL (KERBER & AUNG 1995, 1999), and for *Lr23* on the 2DS chromosome from *Aegilops tauschii* (NELSON *et al*. 1997). Susceptibility loci have also been identified in oats, enhancing the spread of crown rust (WILSON & MCMULLEN 1997).

However, susceptibility genes affecting APR were not identified until WORLAND and LAW (1992) examined APR to yellow rust in the wheat line Hobbit 'sib'. Examination of a monosomic series of Hobbit 'sib' identified chromosomes that contributed to field, yellow rust resistance (i.e. chromosomes 1A, 2A, 4A, 2B, 5BS-7BS, 6B and 2D), but also chromosomes that reduced the level of yellow rust resistance (i.e. chromosomes 3B, 4B, 5BL-7BL, 4D and 5D). In this paper we report on the characterisation of a number of mutants selected for enhanced field resistance to yellow rust in the wheat line Hobbit 'sib' (WORLAND & LAW 1992) and Guardian (BOYD & MINCHIN 2001), and one Guardian mutant showing increased susceptibility.

The generation and characterisation of mutants in Hobbit 'sib'

Mutants were generated in the wheat line Hobbit 'sib' using fast-neutron bombardment (M1). These mutants were assessed in the field for yellow rust resistance as adult plants from the M_2 generation onwards. A number of lines were selected as showing enhanced resistance to yellow rust compared to Hobbit 'sib', and here we present six lines that have given repeatable results in subsequent field and greenhouse, adult plant tests. The increased resistance in all lines, except one, did not appear to be race-specific, the resistance being maintained against more than one race of *Puccinia striiformis* f.sp. *tritici*.

In addition to the enhanced resistance to yellow rust some of these mutant lines also showed increased resistance to other biotrophic fungal pathogens of wheat. Four mutant lines showed partial resistance to brown rust, while one line showed increased resistance to powdery mildew. Mapping programmes are currently underway to identify the mutant loci contributing to the altered resistance phenotype for each disease, and it will be interesting to see if mutant loci for each disease map to the same location.

As the mutant lines were selected in the field for enhanced resistance to yellow rust, greenhouse seedling tests were carried out to determine whether the mutant phenotypes expressed at seedling growth stages. For three of the mutants enhanced yellow rust resistance could be detected at seedling growth stages.

The generation and characterisation of mutants in Guardian

Mutants were generated in the wheat line Guardian using gamma-radiation (200 Grays). These mutants were assessed in the field for yellow rust infection as adult plants from the M_4 generation onwards. A number of lines were selected as showing an altered yellow rust disease phenotype compared to Guardian. Here we report on four lines that were more resistant and one which was more susceptible to yellow rust.

The phenotype of the five mutants remained consistent in repeat field and greenhouse adult plant tests and did not appear to be race-specific, being maintained against more than one race of *P. s.* f.sp. *tritici*. In greenhouse, growth stage-specific tests the enhanced resistant phenotype was expressed at different stages of development in each mutant. A study of infection characters that contribute to overall disease levels showed that the enhanced resistant phenotype was only seen after colonisation of the plant. Significant differences between the mutants and Guardian were only observed for percentage sporulating (the percentage of inoculated leaf tissue producing sporulating colonies) and spore production (the number of spores produced per cm^2 of inoculated leaf tissue). The mutations responsible for the altered infection phenotypes therefore appear to involve plant genes that interact with the pathogen at later stages of pathogen development. As with Hobbit 'sib', some of the mutant lines also showed increased resistance to brown rust and/or powdery mildew. These mutant loci are currently being mapped.

Identification of deletions within the Hobbit 'sib' mutants

As these mutants were made by fast-neutron bombardment it was assumed that the altered disease resistance phenotypes could be due to deletions in the genome. To identify deletions RFLP markers and RDA clones were isolated and hybridised against genomic DNA from each mutant line by HOWIE (1997). Probing with DNA markers identified deletions in all the mutants tested.

Segregation of the Hobbit 'sib' mutant phenotypes

Crosses were made between three of the mutants and Hobbit 'sib'. F_2 populations were screened with the DNA markers that had identified deletions in each mutant to test for segregation of the deletion with the mutant phenotype. In only one mutant was a deletion found to cosegregate with the mutant phenotype. Therefore, these mutants either contain additional, unidentified deletions responsible for the mutant phenotype, or the mutation does not involve a large, readily detectable deletion.

Segregation of the enhanced yellow rust resistance indicated in one mutant line the phenotype was due to a single, dominant mutation, while in another mutant two or more mutation events were responsible for the enhanced resistance phenotype.

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