



Characterisation and deployment of scald (*Rhynchosporium secalis*) resistance in barley

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Abstract

Scald, caused by *Rhynchosporium secalis*, is the most damaging foliar pathogen of barley in southern Australia. The fungus is highly variable and historically this has caused significant problems for breeders seeking durable resistance in barley varieties. The fungus has also been difficult to handle in the laboratory and resistance tests have generally not been consistent.

This paper presents some early results from an examination of the genetic relationship between a large collection of single spore scald isolates and a series of barley differential varieties, with the purpose of establishing an identification system for scald resistance genes. This system will be used to assist the deployment of scald resistance genes in breeding lines by identifying resistance genes present in varieties and advanced breeding lines.

Molecular fingerprinting with Amplified Fragment Length Polymorphism (AFLP) markers is being employed as a means of identifying and maintaining the integrity of scald isolates. Molecular markers may also be used to investigate the relationship between isolate genotype and avirulence and to monitor the changing genetic structure of scald populations.

Introduction

Efforts to understand the genetic basis of *Rhynchosporium secalis* (scald) pathogenicity and host plant resistance began more than half a century ago (Ali *et al*, 1976; Brown, 1985; Boyd *et al*, 1987). Although a number of major resistance genes have been identified, the genetics of the barley-scald interaction is not well understood. This paper aims to examine the interaction between 39 diverse scald isolates on 31 barley varieties as a first step in the development of an isolate collection and screening protocol which may be used to identify scald resistance genes in Australian barley breeding populations and provide a service to barley breeders.

The screening method uses rapidly growing single spore isolates which are being characterised using AFLP markers to monitor isolate integrity. A reliable screening protocol utilising a large range of genetically diverse scald isolates is a useful tool for identifying, monitoring and pyramiding both major and minor resistance genes into new cultivars.

Materials and Methods

Single spore isolate cultures

Scald isolates were collected from field sites across Southern Australia. Isolates from the ACT and NSW were kindly provided by Dr Tony Brown. Initial cultures were obtained following surface sterilisation of infected leaves collected in the field and placed on Potato Dextrose Agar plus 1% streptomycin at 16°C with a 12 h photoperiod. After 2-10 days spores were observed and streaked onto Lima Bean Agar (LBA) plus 1% streptomycin. After 24 hours individual germinated spores were placed on individual LBA plates. After 2-3 weeks spores from the colony were spread and when spores covered the plate they were suspended in 2ml of sterile distilled water and dried onto silica gel at room temperature before storage at -20°C.

Seedling inoculation

Stored *R secalis* spores were streaked onto LBA plates and cultured at 16°C, 12 h photoperiod for 2-3 weeks. Spores were suspended in sterile distilled water and diluted to 1×10^6 spores/ml. 100ml of inoculum containing 2 drops of Tween 20 was used to spray 31 x 10cm pots each containing 4 seedlings at the 2-3 leaf stage. Inoculated plants were incubated in the dark at 16°C, 100% relative humidity for 24h and then maintained with a 12 h photoperiod at the same temperature and relative humidity. The first signs of infection were observed after 7 to 10 days and disease reaction was assessed after 11 days and 21 days for isolates having a longer latent period.

Results and Discussion

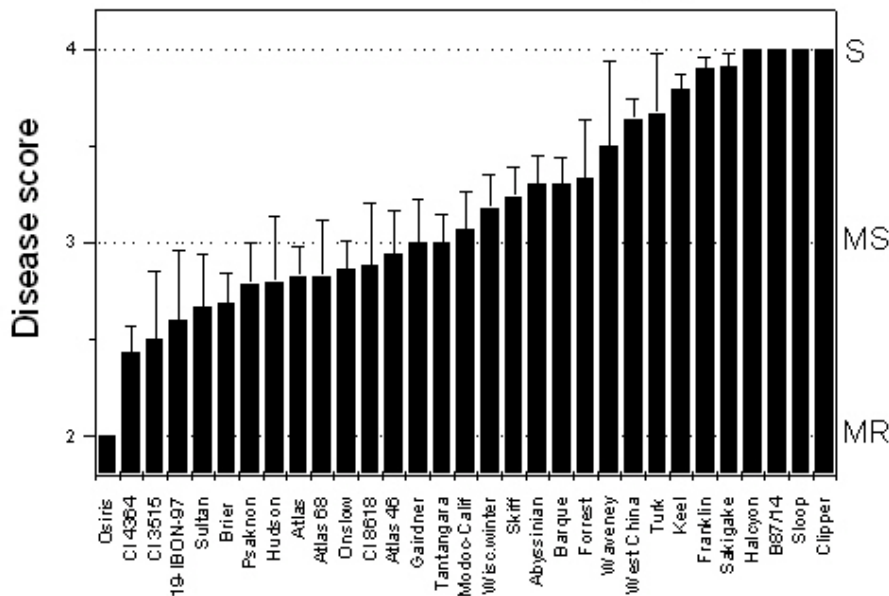
Table 1. Interaction of scald isolates on barley differential varieties. R=resistant (no disease symptoms); MR=moderately resistant (very small lesions (<5% of leaf area) on leaf margin, leaf axil, leaf tip or leaf sheath); MS=moderately susceptible (large leaf lesions between 5-25% of leaf area); S=susceptible (leaf lesions covering >25% of leaf area). Isolates are ordered in increasing virulence from top to bottom. Varieties are ordered in decreasing resistance from left to right.

<i>R secalis</i> isolate	Barley varieties																																												
	Osiris	CI 3515	Sultan	19-IBON-97	Halcyor	Hudson	Waveney	B87/14	Forrest	CI 8618	Turk	Gairdner	Atlas 68	Modoc-Calf	CI 4364	Atlas 46	Wisc winter	Peaknot	Brier	Onslow	Atlas	Tantangara	Abysinniar	Skiff	Barque	West China	Sakigake	Kee	Franklin	Sloop	Clippe														
4	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R											
6	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R										
27	R	R	R	R	R	MR	R	R	R	R	R	R	R	R	MR	R	R	MR	R	R	R	R	MR	-	MR	R	MR	S	MR	MR	S	MR	MR	S	S										
28	R	R	R	R	R	MR	R	R	R	R	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R										
171e	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R									
5F7c	R	R	R	-	R	R	R	R	R	R	R	R	R	R	MR	R	R	-	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR									
30	R	R	R	R	R	R	R	R	R	MR	R	R	R	R	R	S	R	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR									
7	R	R	R	R	R	R	R	R	R	R	R	R	R	R	MR	R	MR	R	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR									
361e	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R								
332d	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R								
341c	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R							
29	R	R	R	R	R	-	R	S	R	R	R	R	R	R	S	R	R	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R							
5B7b	R	R	R	-	R	R	R	R	R	R	R	R	R	R	R	R	R	-	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR							
1	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R							
247b	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R						
168d	MR	R	R	R	R	R	R	R	R	R	R	MR	MR	R	R	MR	R	R	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR						
10	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R						
56	R	R	R	R	R	-	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R					
331a	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R					
3C4b	R	R	R	-	R	R	R	R	R	R	R	R	R	R	MR	R	R	-	S	S	S	MR	-	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR						
139a	R	R	R	R	R	R	R	R	R	MR	R	R	MR	R	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R					
34	R	R	R	R	R	R	-	R	R	R	R	R	R	R	MR	R	R	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR				
262d	R	R	R	R	R	R	MR	R	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R				
5	R	R	R	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R				
100e	R	R	MR	R	R	R	R	R	R	R	-	R	S	R	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S				
332e	R	R	R	R	R	R	-	R	R	-	R	MR	R	R	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R			
4R18b	R	R	R	-	R	R	R	S	R	R	-	R	MR	MR	R	R	-	S	S	S	MR	-	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR			
102f	R	R	R	R	R	R	R	R	R	R	R	R	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R			
32	R	R	R	R	R	R	R	R	R	S	R	MR	R	R	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R		
9	R	R	R	MR	R	R	R	R	R	MR	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R		
20	R	R	R	R	R	R	R	R	R	MR	S	S	MR	R	R	-	S	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	
2	R	MR	R	MR	R	R	R	R	R	R	-	R	R	S	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S		
169d	R	R	R	MR	R	R	R	R	R	MR	S	R	-	R	MR	-	R	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	
6R4a	R	R	R	R	S	MR	S	MR	R	S	R	MR	MR	MR	S	R	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	MR	
84c	R	R	R	R	R	R	R	R	-	R	-	R	S	R	R	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
98e	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
8	R	R	R	R	S	MR	S	S	MR	R	S	R	MR	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
H2.5	R	R	MR	S	R	R	R	R	R	S	-	R	S	S	S	-	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
332a	R	MR	MR	R	S	S	S	S	S	R	S	S	S	S	MR	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Table 1 illustrates the complexity of interactions between host and pathogen. Each isolate has a unique pattern of virulence across the 31 differential varieties which was repeatable in most cases. Varieties were arranged in order of decreasing resistance and scald isolates arranged in order of increasing virulence following calculation of a mean value based on scores of 1, 2, 3 and 4 for interactions rated R, MR, MS and S, respectively. By choosing suitable isolates it should be possible to identify the presence of absence of resistance genes in segregating breeding populations.

To attempt to assess levels of partial resistance, variety-isolate combinations which showed no symptoms (R) were removed from the analysis and a measure of disease development was calculated by allocating scores of 2, 3, and 4 to MR, MS and S reactions, respectively, and calculating the mean across isolates to provide a "disease score" for each variety. These data are presented in Fig. 1.

Fig. 1. Disease score of varieties after inoculation with isolates which induce scald symptoms



There are significant differences in the extent of symptoms exhibited by the different varieties. Varieties such as Clipper, Sloop, B87/14 and Halcyon had a disease score of 4 (S) against all isolates capable of inducing disease symptoms on them. Others, such as 19-IBON-97, C3513 and CI4364 had scores between 2-3 (MR-MS) whilst Osiris, which showed symptoms with only a single isolate, had the lowest disease score of 2 (MR). These scores may indicate differences in the genetic capacity of different barley genotypes to resist the *Rhynchosporium* fungus as seedlings

We are also developing methods to assess scald resistance in adult plants using *in vitro* inoculation of mature detached leaves. Although seedling assay scores and field disease levels have been shown to be closely correlated in some studies (Abbot *et al*, 1991) this correlation breaks down for varieties such as Franklin and Keel which have good levels of adult plant resistance while being susceptible to many scald isolates as seedlings (Table 1). A combination of seedling and adult plant tests will form the basis of a service to assist barley breeders select for scald resistance.

Acknowledgments

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References

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