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Host status and genetic analysis of blast (*Magnaporthe oryzae*) resistance in barley

Carol Nunurai Bvindi



Masters of Science Research Thesis
Submitted to
Wageningen University and Research Centre
In partial fulfillment of MSc Plant Sciences Degree
Specialisation: Plant Breeding and Genetic Resources
January – July 2010

Supervisor: Dr. Reza Aghnoum

Examiners: Dr. Rients E. Niks
Dr. Gert Kema



Acknowledgements

My sincere gratitude goes to Reza Aghnoum, for his day to day supervision, ideas, helping hand in the experiments and in writing this report; to Rients Niks for being always available to help; to Joao Nunes Marciel for teaching all I needed to know about *Magnaporthe oryzae*; to Gert Kema for ideas in the project and sourcing the isolates, to CIRAD (France) and Embrapa (Brazil) for providing the isolates.

Summary

Magnaporthe oryzae is a hemi-biotrophic fungus with a wide host range, including many members of the poaceae family (Zellerhoff et al., 2008, Inukai et al., 2006). Here a study is reported where the host status of barley to *M. oryzae* isolates from wheat and rice and its resistance to the wheat isolate is investigated. All the tested wheat and the rice isolates of *M. oryzae* were pathogenic to the majority of barley tested accessions, though showing a high degree of pathotype specificity. Disease tests with a Brazilian wheat isolate (PY67.1) revealed one line which was completely resistance, a few partially resistant and many susceptible lines. Inoculation of an F₂ population derived from a cross between the resistant accession CGN02587, an East African barley landrace, and Vada, a modern West European barley cultivar, showed a normal distribution of the disease severity scores suggesting that many genes were responsible for the resistance of CGN02587.

Two Recombinant Inbred Lines (RILs) populations (Vada x SusPtrit and L94 x Vada populations) in which the parental lines show a differential response to a wheat isolate of *M. oryzae* were evaluated for disease severity at seedling stage and one of the population (L94 x Vada) at adult stage. One Quantitative Trait Locus (QTL) conferring blast resistance was mapped on chromosome 7H in the two populations at seedling stage and two QTLs were mapped on chromosome 2H and 7H at adult stage. These QTLs were growth stage dependent.

To further investigate barley's resistance to the wheat isolate, association mapping was done on a set of 148 modern European two-row spring barley cultivars. Associations between two markers on chromosome 5H and 7H were found with the phenotypic blast resistance data of seedling stage. The two markers did not coincide with QTLs mapped in this study both at seedling and adult stage, however the marker on chromosome 7H did coincide with a QTL reported by Inukai et al., 2006 for barley's resistance to rice isolate of *M. oryzae*

Histologically evaluating three barley accessions which showed complete resistance, partial resistance and susceptible phenotypes to the wheat blast isolate showed that in the resistant accession the pathogen growth is curbed in the epidermal cells by epidermal and mesophyll Hypersensitive Reaction (HR), in susceptible phenotypes growth of the fungus proceeds to the mesophyll cell with the plant hardly responding to the growth of the fungus and partially resistant phenotypes shows a somewhat intermediate response.

This study has succeeded in demonstrating that barley is indeed a host to *M. oryzae* isolate from wheat and rice, identified genomic regions associated with barley's resistance to an isolate of *M. oryzae* from wheat and elucidated the mechanism of this resistance histologically. This report presents the first step in breeding for blast resistance in barley, and these results are useful in creating blast resistance cultivars in case a blast epidemic occurs in barley.

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Chapter 1

1. Introduction

Wheat blast disease is caused by the *Triticum* pathotype of the filamentous ascomycete fungus *Magnaporthe oryzae*. It is a devastating pathogen of wheat, only important in Brazil though it poses economic threats to the global wheat production and the food security of more than half of the world's population (Wilson and Talbot 2009). *M. oryzae* has been defined as a different species separate from *Magnaporthe grisea* based on phylogenetic analysis where gene trees were inferred using portion of actin, beta-tubulin and calmodulin genes (Couch and Kohn 2002,). However, these two species cannot be distinguished morphologically. Isolates of the fungus pathogenic to wild grasses including the wild crabgrass of the *Digitaria* genus were found to belong to the *Magnaporthe grisea* species whilst those isolates pathogenic on cultivated cereals were found to belong to the *Magnaporthe oryzae* species (Couch and Kohn 2002). Thus isolates of *Magnaporthe oryzae* are able to cause the blast disease in several species of the *Poaceae* family that includes cereals among others wheat, rice and barley (Inukai et al., 2006; Zellerhoff et al., 2008).

1.1 Distribution and economic importance

Wheat blast was first reported as a significant wheat disease in 1986 in the Parana state of Brazil (Igarashi 1990); from then on it spread to other 27 wheat growing states in the North and West of the Parana state. Currently it is present in all wheat growing regions of Brazil continues to affect wheat production there. It has been reported to cause losses of up to 50% whenever susceptible genotype is cultivated in country (Murakami et al., 2000, Oh et al., 2002). The pathogen has been shown to significantly reduce yield, grain weight, and milling quality as chemical control methods are not effective and resistant cultivars are seldomly reported in that country (Greer and Webster, 2001). Furthermore wheat is an important crop in Brazil, as staple food and an important part of the daily diet of millions of people in that nation (Prestes et al., 2007). Thus this pathogen causes has great impact on the economy and social well being of nation of Brazil. However the disease has not yet spread to other wheat-growing regions of the world though it remains a potential threat (Greer and Webster, 2001; Prestes, 2007).

1.2 Disease cycle

M. oryzae is a facultative fungus which can also grow on non living substrate. It affects cereals at virtually all stages of development. The fungal life-cycle on its primary host- rice is well-studied, but a little is comparatively known about disease progression on hosts other than rice (Skamnioti and Gurr 2009). Its life cycle is completed through spore production and it can reproduce sexually and asexually. Asexual reproduction is through conidia spores disseminated by air. Once it lands on the leaves it adheres to it through the release of an adhesive from an apical compartment at the tip of the spore, the so called spore tip mucilage (STM). The spore germinate, a germ tube grows from the spore and later develops in a dome shaped appressorium. The appressorium develops a penetration peg which serves to carry the pathogen through the surface of the leaf into the underlying epidermal cells. After penetration, the fungus develops another distinct structure- an infection hyphae which is also known as the secondary hyphae. This hyphae is invasive and it grows intracellularly, invading adjacent epidermal cells as well as underlying mesophyll cells throughout the leaf. Subsequent colonization of the leaf cells produces disease lesions which release thousands of new conidia. These new conidia spread to new plants by dew splash or air and thus reinitiating the disease cycle. Sexual reproduction occurs when two strains of opposite mating type meet and form a perithecium in which ascospores are formed. Once released, ascospores can develop appressorium and infect host cells following the afore-mentioned cycle (Howard and Valent 1996, Dean et al., 2005; Skamnioti and Gurr 2009). The disease is a polycyclic and is highly dependent on the favourable conditions for the fungus to grow. The fungus overwinters as conidia or mycelium on seeds, crop residues, other winter cereals or grass hosts. Its favourable conditions are high relative humidity about 80%, high temperature around 24-28 °C and long periods of leaf wetness (Howard and Valent 1996).

1.3 *M. oryzae* infections on barley

Compatibility of *M. oryzae* isolate from rice and barley has been reported years ago (Thomas 1940; Sato et al., 2001), however infection of barley fields were reported much later in 1979 in Japan (Sato et al., 2001). Recently infection of barley by *M. oryzae* from rice has been reported in Japan where barley and rice are rotated in the same fields (Sato et al., 2001), epidemics of the disease have also been reported in

California (Greer and Webster 2001) and in Thailand (Sato et al., 2001). In Brazil spike infection that's showed similarity to those of wheat (*Triticum aestivum*) and rice (*Oryzae sativa*) blast were reported in the barley field in 2001 and 2002 cropping season (Lima and Minella 2003). Yield losses caused by the fungus on barley have not yet been reported, however it is anticipated that the fungus may poses a potential economic threats on barley similar to those the fungus cause on wheat and rice, where yield loss can reach 10 million tonnes per year (Kongaprakhon et al., 2009). Thus there is need for breeding for blast resistance in barley to counteract these threats. This entails genetic analysis of the different kinds of resistances to *M. oryzae* found in barley. Barley is the world's fourth most important cereal and it is grown for fodder, human consumption and brewing of beer.

1.4 *M. Oryzae* resistance in barley

The two major types of resistance (complete and partial resistance) have been reported in barley's responses to *M. oryzae* isolates from rice; with complete, race-specific resistance conferred to by PHR-I reported by Yaegashi (1978) and *RMo1* reported by Inukai et al. (2006). Partial and quantitative resistance conferred by four QTLs reported by Sato et al. (2001), twelve QTLs to three different blast isolates reported by Chen et al. (2003) and three other QTLs reported by Inukai et al. (2006). This shows that studies of barley's resistance to *M. oryzae* are limited; furthermore all these reports are based on barley's resistance to *M. oryzae* isolate from rice. No report so far has been presented on barley's interaction to *M. oryzae* isolate from wheat. With the threat the disease poses on barley, it is of high importance to study the host status of barley to the wheat isolates and the genetics of blast resistance in barley.

1.5 Breeding for blast resistance in barley

In breeding for blast resistance in rice, identification of QTLs conferring partial resistance has been proven useful compared to complete major genes conferring complete monogenic resistance. This is mainly due to the fact that complete monogenic resistance is easily broken down because of the race specificity and the rapid change in pathogenicity of the blast fungus (Ribot et al., 2007). Also complete monogenic resistance induces high selection pressure on the pathogen population favouring those pathogens that overcome the resistance by mutation or loss of the corresponding avirulence factor. From the lessons learnt from rice, it is important in breeding for blast resistance in barley to identify QTLs conferring partial resistance to

blast in barley compared to major genes conferring complete monogenic resistance. Partial resistance is more preferred as it is durable because it is controlled by many genes thus not easy to overcome by the pathogen. In this report partial resistance was analysed using two methods which are QTL mapping and association mapping.

QTL mapping involves using full sib mapping population (F_2 , DH, RILs, and backcross populations) to test DNA markers throughout the genome for their likelihood of linkage with a QTL. Basically the mapping population is analyzed in terms of DNA marker genotype and phenotype of interest, and split into genotype groups according to the marker genotype. Means and variances are compared between these groups to determine whether significant differences exist between the groups with respect to the trait of interest. A significant difference indicates that there is a relationship between the DNA marker and the QTL controlling the trait of interest (Young 1996; Collard et al., 2005). The principle of QTL mapping is based on the underlying assumption that linkage disequilibrium (non random association of alleles) exist between the alleles at the marker locus and the alleles at the QTL. This level of LD between loci is influenced by inbreeding, natural selection, mutation rate, the size of the population and physical linkage of loci in segregating populations. Thus segregating populations have the highest ability to map and characterize QTLs (Tanksley 1993).

On the other hand association mapping is a method of mapping quantitative trait loci based on the strength of correlation between the mapped genetic markers and the trait of interest in populations without a simple genetic structure (Yu et al., 2008, Flint-Garcia, 2003). In its simplest form, it involves identifying markers with significant allele-frequency differences between individuals with the phenotype of interest and a set of unrelated control individuals. A statistical association between genotypes at a marker locus and the phenotype is usually considered to be evidence of close physical linkage between the marker and the phenotype (Pritchard et al., 2000). Association studies test whether an allele occurs at high frequency in one particular phenotype than in the other (for example is the allele frequency high in affected than in unaffected individuals) thus, it involves population correlation rather than co-segregation within a family which linkage analysis rely on (Lander and Schork 1994).

Association mapping like QTL mapping relies on linkage disequilibrium (LD) which is also known as gametic phase disequilibrium. However, LD will decay in a population and the rate is determined by genetic distance, the number of generations

since it arose population structure and recombination. In an unstructured population (random mating populations) meiotic and recombination events are high, only the genetic markers in linkage disequilibrium with the trait of interest will associate with the trait. Thus, LD mapping offers high resolution/ fine mapping of markers associated with the trait of interest. Furthermore, application of the method to unstructured populations, the applicability of the results to a wider germplasm and its complementarity to the traditional linkage mapping are the other advantages the method offers in QTL analysis (Flint-Garcia, 2003, Mackay and Powell 2007, D'Hoop et al., 2008).

1.6 Research objectives

This study was aimed at investigating the host status and the genetics of the resistance of barley to wheat (*Triticum*) infecting form of *M. oryzae*. This research basically presents the first step in breeding for blast resistance in barley where the host status of barley to *M. oryzae* isolate from wheat is elucidated; the genomic regions in barley associated with the resistance to the disease are identified and the mechanism of resistance are observed histologically. The following specific objectives were addressed:

1. Determination of the host status of barley to isolates of *M. oryzae* collected from rice and wheat.
2. Mapping QTLs for quantitative resistance of barley to blast in L94 x Vada and in Vada x SusPtrit RILs populations.
3. Association mapping of blast resistance in modern two-row spring barley cultivars.
4. Genetic analysis of the complete resistance found in CGN02857, using F2 population derived from CGN02857 x Vada.
5. Study the histopathology of barley-*Magnaporthe* interaction in susceptible, partially and completely resistance interactions.

Chapter 2

Materials and methods

2.1 Planting material

A collection of 110 accessions of barley was used in evaluating the host status of barley to wheat and rice isolates of *M. oryzae*. The list of the accessions included diverse lines grown in Europe, experimental lines and landraces collected from different parts of the world. For the isolate specificity experiment, nineteen barley accessions from the 110 accession list which showed different responses (from resistant to susceptible) were tested with ten different *M. oryzae* isolates from wheat and three from rice. Rice and wheat cultivars were added to the isolate specificity experiments to act as controls.

For genetic analysis of blast resistant at seedling stage, a set of 103 Recombinant Inbred Line populations (RILs) derived from a bi-parental cross of L94 x Vada (LnVa) (Qi et al., 1998) and a set of 152 RILs derived from Vada x SusPtrit (VaSu) (Jafary et al., 2006) were used. At adult plant stage QTL analysis was only done on the LnVa mapping population. Molecular linkage maps for these RILs populations were previously generated and have been used in QTL mapping of leaf rust (*P. hordei*), and powdery mildew (Qi et al., 1997, Qi et al., 1998; Aghnoum et al., 2010). L94 is an Ethiopian landrace, with black and naked seeds, and it shows partial resistance to several wheat isolates of *M. oryzae*. Vada is a commercial West European cultivar, with white and covered seeds, previously released by the Department of Plant Breeding, Wageningen University and Research Centre, and is susceptible to blast. SusPtrit is an experimental line that is susceptible to several inappropriate rust fungi (Jafary et al., 2006) and is partially resistance to blast.

For association mapping studies, a collection of 148 modern two row spring cultivars referred to hereafter as ‘The Kraakman series’ were analysed. These are homozygous diploid lines created by inbreeding or by doubling haploids and represent a large part of European germplasm used for the past two decades. A genetic map for this population was already established by Kraakman et al. (2004) and this population has been previously used in marker-trait association studies of yield, yield stability, heading date, plant height, disease resistance and morphological traits (Kraakman et al., 2004, 2006).

To analyse the complete resistance found in CGN02857, an F₂ population derived from the cross between CGN02857 and Vada were grown and used in a seedling test to determine the qualitative or quantitative nature of the resistance. CGN02857 is a line from East Africa and it showed complete resistance to blast in a previous barley screen of 110 lines.

For the study of the interaction of barley and *M. oryzae* at cellular level at seedling stage, three barley lines (CGN02857, Vada and L94) differing in their response to *M. oryzae* were evaluated histologically. CGN02857 is completely resistant, L94 is partially resistant and Vada is susceptible to blast.

2.2 Pathogen isolates.

A *Tritici* isolate of *M. oryzae* (PY67.1) collected from Londrina-Paraná state of Brazil and rice isolate GY0011 collected from Combi, French Guyana were used in the host determination experiment. For the isolate specificity experiment ten wheat isolates (PY67.1, PY30.1, PY06001, PY0629, PY6037, PY6047, PY19.1, PY47.2, PY22.2, and PY41.2) and three rice isolates (JP9, ML25, and PH14) were used. For histological analysis, QTL and association mapping, the wheat isolate PY67.1 was used. These isolates were maintained on dried filter paper and propagated on oatmeal agar. The wheat isolates were provided by Embrapa, Brazil and the rice isolates were provided by CIRAD, Montpellier, France. Full details including the collection date, country, organ, and the cultivar from which the isolates were collected are shown in the table 1.

2.3 Inoculum preparation

The *M. oryzae* isolates were grown for 15 days on oatmeal agar at 25°C under a 16: 8 hour light: dark cycle. Scratching the fungus was done after 15 days and at exactly three days before inoculation to stimulate spore (conidia) formation. Conidia were harvested by flooding the plates with 5 ml of sterile inoculation solution which contained 0.25% gelatine and 0.01% Tween 20. Following flooding the conidia was scraped gently from the surface using a sterile glass rod and filtered through a sterile cloth. The spore density was adjusted to 2 x 10⁵ conidia per ml by addition of the inoculation solution.

Table 1: Details of *M. oryzae* isolates used in this study.

Name	Collection date	Country of origin	Organ collected from	Host
PY06001	6-05-2006	Coromandel, Brazil	Leaves	<i>Triticum aestivum</i>
PY0629	8-3-2006	Coromandel, Brazil	Rachis	<i>T. aestivum</i>
PY47.2	2008	Christalina, Brazil	Spikes	<i>T. aestivum</i>
PY 22.2	10-8-2008	Palontina, Brazil	Spikes	<i>T. aestivum</i>
PY 41.2	2008	Burutis, Brazil	Spike	<i>T. aestivum</i>
PY 30.1	2007	PADPPF, Brazil	Spikes	<i>T. aestivum</i>
PY 67.1	2008	Londrina–PR, Brazil	Spikes	<i>T. aestivum</i>
PY19.1	10/8/2008	Unai – MG, Brazil	Spikes	<i>T. aestivum</i>
PY6037	3-8-2006	Goiânia, GO, Brazil	Rachis	<i>T. aestivum</i>
PY6047	3-8-2006	Goiânia, GO, Brazil	Rachis	<i>T. aestivum</i>
GUY11	6-1-1978	Combi, Guyana France	Leaf	<i>Oryzae sativa</i>
ML0025	9-1-1986	Niema ,Mali	Neck	<i>O. sativa</i>
JP0009	*	Japan	*	<i>O. sativa</i>
PH0014	7-8-1980	IRRI, Philippines	Leaf	<i>O. sativa</i>

*- Missing data.

2.4 Inoculation and disease evaluation

2.4.1 Seedling stage

Barley plants were grown in a 40-well plastic tray measuring 7 x 7 x 6 cm. Four seeds of each line were sown in each well and wheat or rice lines were also added in each tray to act as controls. The seedlings were grown in spore-free greenhouse compartment at 25°C under artificial light. Two week old seedlings were inoculated by spraying onto the leaves with 14 ml of conidial suspension containing 2×10^5 spores per ml per each tray. The inoculated plants were placed in clear plastic bags to maintain a water-saturated atmosphere and placed in a greenhouse at 25°C covered with black plastic bags. After 12 hours the black plastic bags were removed, and after

24 hours the clear plastic bags were later on removed. The plants were then placed in a high humidity (80%) greenhouse at 26°C. Disease severity was scored at 5 days after inoculation based on the scoring scale suggested by Oh et al. (2002) which is 0=no visible reaction, 1=brown pinpoint spots, 2= small brown lesions, 3= intermediate lesions, 4=large lesions covering 50-50% of the leaf, 5=large coalesced lesions resulting in complete blighting of the leaf blades.

2.4.2 Adult plant stage

For the adult plants evaluation, the RILs of LnVa mapping population were grown in 14 cm pots in a spore free greenhouse to adult plant stage. At anthesis, the plants were inoculated with a wheat blast isolate (PY67.1) by spraying the conidial suspension at a spore density of 2×10^5 spores/ml. The inoculated plants were put in a growth chamber with temperature adjusted to 25°C under saturated moisture for 24hours. Thereafter the plants were put and maintained in the green house with temperature adjusted to 26°C and relative humidity of 80% under artificial light. Disease severity was scored at 10 days after inoculation. The following scale was used: 0 = healthy, absence of symptoms; 1 = light, limited dark lesions at the base or along the rachis or light-coloured elliptic lesions of the external parts of the glumes but without spikelet death; 2= light to moderate, death of one to seven isolated spikelets; 3 = moderate, groups of dead spikelets covering 1/3 to 2/3 of the area of the spike; and 4 = severe, death of the whole spike (Cardoso et al., 2008).

2.5 QTL analysis

Wheat blast disease phenotypic data scored at seedling in two mapping populations LnVa and VaSu and at adult plant stage in LnVa were combined with the already available bi-parental maps and was analysed with a computer software package MAPQTL version 6 (Van Ooijen et al., 2004). Interval mapping (Lander and Botstein, 1989) was done to estimate the map location, LOD score and the phenotypic effect of the potential QTL in terms of the percentage variance. Peak markers from the interval mapping were taken as co-factors for running multiple QTL mapping (MQM) programme (Jansen and Stam 1994) until a stable LOD profile was reached. A significant QTL was declared at a LOD value ≥ 3 . The detected QTLs were taken from the individual maps to the integrated map and QTLs with LOD-2 overlapping were declared as the same QTL.

2.6 Association mapping

Association mapping studies were done using an AFLP marker map generated by Kraakman et al. (2006) and the average of phenotypic data collected from the five days after inoculation of the 'Kraakman series' with wheat isolate PY67.1 of *M. oryzae*. In the association analysis, simple linear regression of the response of the trait on the AFLP was done using the software GGT 2.0 (Van Berloo 2007). The corresponding correlation coefficient(r) and p-value were used to declare significant marker-trait associations. To control for multiple testing, False Discovery rates (FDR) expressed as q-values were calculated. By definition FDR is the expected proportion of true null hypothesis in a class of rejected null hypothesis, in its simplest form it is when marker-trait associations are declared significant yet no associations exist in reality. To further assess marker-trait associations, association profiles were created by plotting p-values for marker-trait correlation against chromosome position; these graphically show the LD region around associated markers and help in assessing the credibility of a marker-trait association. Furthermore we assessed marker-trait associations that have been reported in literature to check the associations that we found.

2.7 Bulk segregant analysis (BSA)

Bulk segregant analysis is a rapid and cost effective procedure for identifying markers in specific regions of a genome (Michelmore et al., 1991). An f_2 population derived from a cross between CGN02857 and Vada was phenotyped to identify individual plants which are extremely resistant and extremely susceptible. DNA was extracted from the leaves of the plants using the procedure mentioned in 2.8. Two contrasting DNA bulks were prepared one from the extremely resistance and the other from the extremely susceptible individuals. Marker analysis on these two DNA bulks to identify polymorphic markers that distinguish them is however still in progress and thus not much can be mentioned in this thesis report.

2.7.1 DNA isolation

Two leaf discs of about 3cm of each line were harvested from two weeks old F_2 seedlings were pooled in a Micronic[®] deepwell tube; of a 96 deep-well plate and 2 stainless steel balls were added to each tube. The leaf discs were grinded by the Retsch apparatus in 300 μ l Agowa[®] Lysis buffer P from the AGOWA[®] Plant DNA Isolation Kit and 0,5 μ l RNase (2 mg/ml). After grinding the plates were incubated at

65°C in a water bath for 30 minutes after which the plates were centrifuged for 5 minutes at 3000 rpm. 200µl of the green supernatant was pipetted out to a 96 Kingfisher deep well plate with 520µl binding buffer and magnetic beads (60 µl). DNA isolation proceeded following the Maxi protocol of the Kingfisher® (Thermo lab systems, Finland). The following steps were carried out by the Kingfisher® : DNA was bound to the beads, two successive washing steps were carried out, washing step 1 was with Agowa® wash buffer 1 and washing step 2 was with Agowa® wash buffer 2 and finally the DNA eluted in elution Agowa® elution buffer.

2.8 Histological analysis barley infection by *M. oryzae* at seedling stage

Disease symptoms at seedling stage on the three afore-mentioned lines were microscopically evaluated at 14, 24 and 48 hours post inoculation (hpi) with *M. oryzae* isolate PY67.1 from wheat. Plants were grow and inoculated as mentioned above in section 2.4.1. Leaf segments of about three centimeters of the inoculated plants were harvested at above mentioned post inoculation time intervals. The harvested leaves were subjected to three different staining method which are trypan blue, Uvitex and 3, 3'-diaminobenzadine (DAB) staining. For trypan blue staining the harvested leaves were placed in acetic acid-ethanol(1:3v/v) for at least 30 minutes and later incubated in lacto-phenol with 0.005% trypan blue at 65°C for one hour. The leaves were cleared with saturated chloral hydrate (5:2 w/v) for at least 24 hours after which they were embed in glycerol and fungal structures viewed under the light microscope. For Uvitex staining, harvested leaves were prepared as described by Chen et al. (2010) and viewed under epi-flourescence microscopy. For DAB staining, procedure was followed as described by Huckelhoven et al. (1999). For each staining procedure and time interval one leaf of each line were analyzed and two biological replications were done for the entire experiment. All the interaction sites per genotype were inspected and scored according to the method described by Tufan et al. (2009) which is **A**- appressorium formation with no staining or fluorescence of plant cells beneath it, **B**-invasive hyphae formation with staining or auto-flourescence of the attacked epidermal cells, **C**-secondary hyphae formation with staining of regular shaped mesophyll cell and **D**- secondary hyphae spanning multiple cell associated with staining or strong auto-flourescence of collapsed mesophyll cell.

Chapter 3

Results

3.1 Host status of barley to *Tritici* and *Oryzae* isolates of *M. oryzae*

The host status of barley to *M. oryzae* isolates of wheat and rice was determined by testing a set of 110 barley accessions with one wheat isolate PY67.1 and one rice isolate GUY11. Of the 110 accessions tested with the wheat isolate, 52 accessions were susceptible (score 4 and 5), 55 lines were partially resistant (score 2 and 3) and only one line was completely resistant (score 1) (Fig 1). With the rice isolate, 37 lines were susceptible, 57 partially resistant and 14 completely resistant (Fig 1). The susceptible lines produced many green water soaked lesions which later enlarged and coalesced with time resulting in shrivelling of the whole leaf. The partially resistant lines had discrete to confluent brown coloured pinpoint lesions and the completely resistant lines produced no visible lesions only small pinpoint brown necrotic spots. There was evidence of pathotype specificity of the resistance of barley as two lines showed resistance to wheat isolate and susceptible to the rice isolate. Five lines were resistant to the rice isolate and susceptible to the wheat isolate.

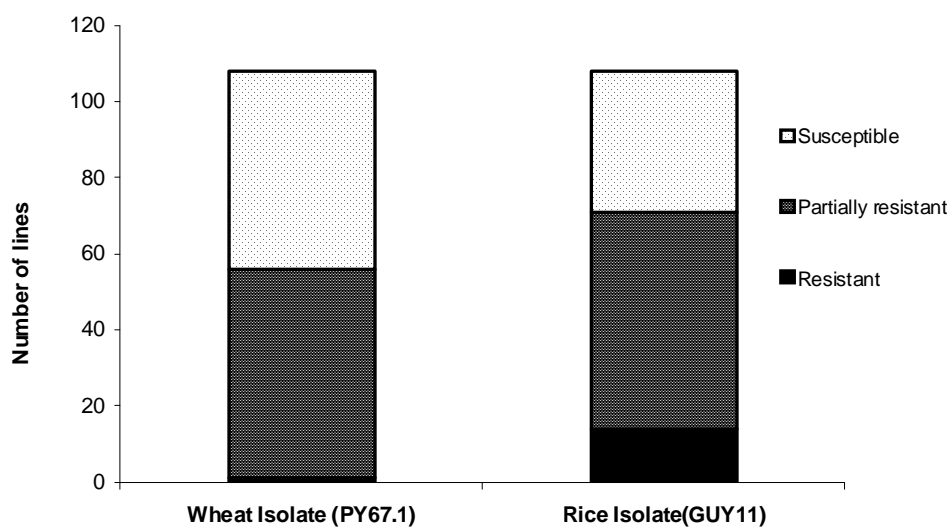


Figure 1: Phenotypic response of barley lines to wheat isolate PY67.1 and rice isolate GUY11 of *M. oryzae*.

Furthermore we tested isolate specificity of barley's resistance to *M. oryzae* using ten isolates collected from wheat, three from rice and nineteen barley selected from 110 list initially used in the host status experiment. The pathogenic reactions of these

barley accessions to the different isolates from rice are presented in table 2a and from wheat in table 2b. All the wheat and rice isolates tested were pathogenic on barley, and thirteen lines responded uniformly producing constant infection types to all the wheat isolates. For example, Vada, Steptoe and Morex were all susceptible to all the isolates, L94, SuspTrit, CGN22 and Prominent were partially resistant, and CGN02857 was completely resistant to all the tested isolates. The rest of the lines were resistant to some wheat isolates and susceptible to some. For the response to the rice isolate ten accessions responded uniformly to all the isolates tested and only eight had differential response. All wheat isolates were pathogenic on wheat cultivars Anahuac and Renan which acted as controls and all the rice isolates were pathogenic to rice accessions CO39 and Sariceltik which also acted as controls to rice isolate experiments. Our results showed that barley is a host to both *Tritici* and *Oryzae* isolates of *M. oryzae*. Furthermore they indicate pathotype and isolate specificity of the resistance of barley to *Tritici* and *Oryzae* isolates of *M. oryzae*.

Table 2a: Disease severity scores of barley and wheat lines inoculated with different *M. oryzae* isolates form rice.

Name	PH14	JP9	ML25	Note:
Nure	2	3	4	0= no visible reaction,
Tremois	2	1	1	1=Brown pinpoint spots,
Susptrit	2	3	3	2= small brown lesions,
Cebada Capa	2	3	2	3= intermediate lesions,
Prisma	4	2	3	4=large lesions covering
Apex	3	5	3	50-80% of the leaf,
DOM	4	5	4	5=large coalesced lesions
REC	4	3	5	resulting in complete
L94	2	3	3	blighting of the leaf
Vada	3	*	3	blades. Inoculated
Steptoe	5	3	5	seedlings were grown in
Morex	2	2	5	the greenhouse at 25°C
Ab 14 Köln	*	*	5	and scored for infection
Prominent	1	2	3	type 5 days post
CGN02424	3	2	4	inoculation.
CGN02857	1	1	1	*- Missing results.
Henni	5	2	5	^a - Rice cultivars as
Meltan	4	3	*	control
CO39 ^a	*	5	4	
Sariceltik ^a	2	2	2	

Table 2b: Disease severity scores of barley and wheat lines inoculated with different *M. oryzae* isolates from wheat.

Name	PY06001	PY0629	PY20.2	PY22.2	PY41.2	PY30.1	PY19.1	PY6037	PY6047
Nure	4	4	5	5	4	4	3	4	1
Tremois	4	3	3	3	3	4	2	2	1
Susptritt	4	2	3	3	3	4	2	2	2
C. Capa	4	3	4	4	4	4	2	3	3
Prisma	3	2	3	2	3	3	1	2	3
Apex	5	3	3	4	3	3	1	1	3
DOM	4	5	5	5	5	4	3	4	3
REC	4	5	5	5	5	4	*	*	*
L94	3	2	2	2	2	2	*	1	1
Vada	5	5	5	5	5	5	5	5	5
Steptoe	4	4	5	5	5	5	4	4	4
Morex	4	5	5	4	4	4	4	5	5
Ab 14 Köln	3	*	*	3	3	3	1	*	1
Prominent	2	1	2	1	2	1	0	1	1
CGN 6	2	2	3	3	*	2	1	1	1
CGN 22	2	3	3	2	2	2	2	2	1
CGN 37	1	0	2	0	1	0	0	0	0
Henni	4	4	5	4	4	4	5	4	4
Meltan	3	3	3	3	3	3	2	2	2
Falat	5	5	5	5	4	3	*	*	*
Renan ^a	2	4	*	0	0	4	0	1	2
Vivant ^a	5	5	5	5	5	5	1	1	1
Anahuac ^a	5	5	5	5	5	5	*	*	*

Note: Infection type 0= no visible reaction, 1=Brown pinpoint spots, 2= small brown lesions, 3= intermediate lesions, 4=large lesions covering 50-80% of the leaf, 5=large coalesced lesions resulting in complete blighting of the leaf blades. Inoculated seedlings were grown in the greenhouse at 25°C and scored for infection type 5 days post inoculation.

*- Missing results.

^a- Wheat cultivars as control.

3.2 QTLs mapped at seedling stage

The common blast symptoms of water soaked leaf lesions developed in the two RIL populations and their parents. L94 (score 2) and SusPtrit (score 3) showed partial resistance reaction with a few small water soaked lesions surrounded by brown necrotic areas around the lesions. On the contrast Vada was very susceptible (score 5), it developed very large green water soaked lesions that covered the entire leaf (Fig 2). The wheat blast phenotypic scores were collected for the three replicates of each population and the average was used for frequency distribution and QTL analysis. In LnVa the disease severity score values of the RILs fell between those values of the parental lines (fig 3); no transgressive segregation was observed, suggesting that the resistance alleles were contributed by one parent and the susceptible alleles by the other. However in VaSu transgressive segregation towards resistant phenotypes were observed suggesting that both parents carry some genes for partial resistance to blast. The phenotypic frequency distribution for the two RILs population did not fall into discrete phenotypic classes, neither was it continuous but on the contrast it was skewed toward the susceptible phenotypes. Using interval mapping and multiple QTL mapping one chromosomal region (QTL) was indentified that contributes to blast resistance at seedling stage, and was mapped on the top of chromosome 7H in both LnVa and VaSu populations. The peak markers on the QTL region were AFLP markers E33M55-508 and E42M51-232 in LnVa and VaSu respectively. The likelihood of odds (LOD) values of the peak markers were 17.14 in LnVa and 17.29 in VaSu populations. Percentage variation the QTL explained between the RIL populations was 54.6% in LnVa and 41.8% in VaSu. An overview of the QTLs is given in table 3.

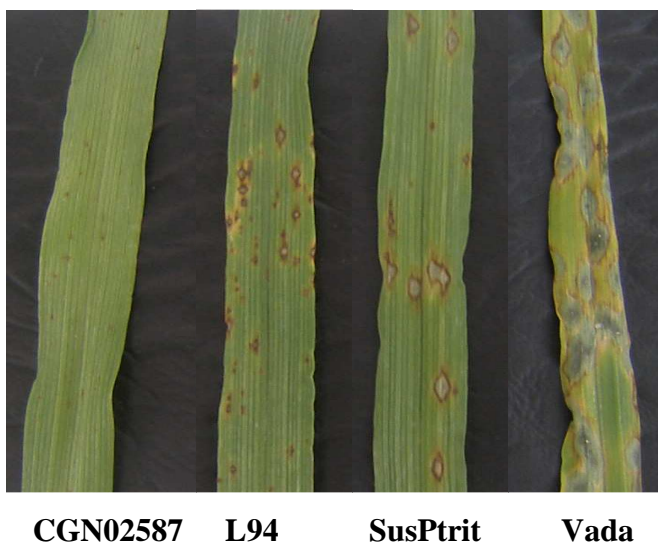


Figure 2: Phenotypes of the barley genotypes CGN02587, L94, SusPtrit and Vada five days after inoculation with the *Tritici* isolate of *M. oryzae* (PY67.1).

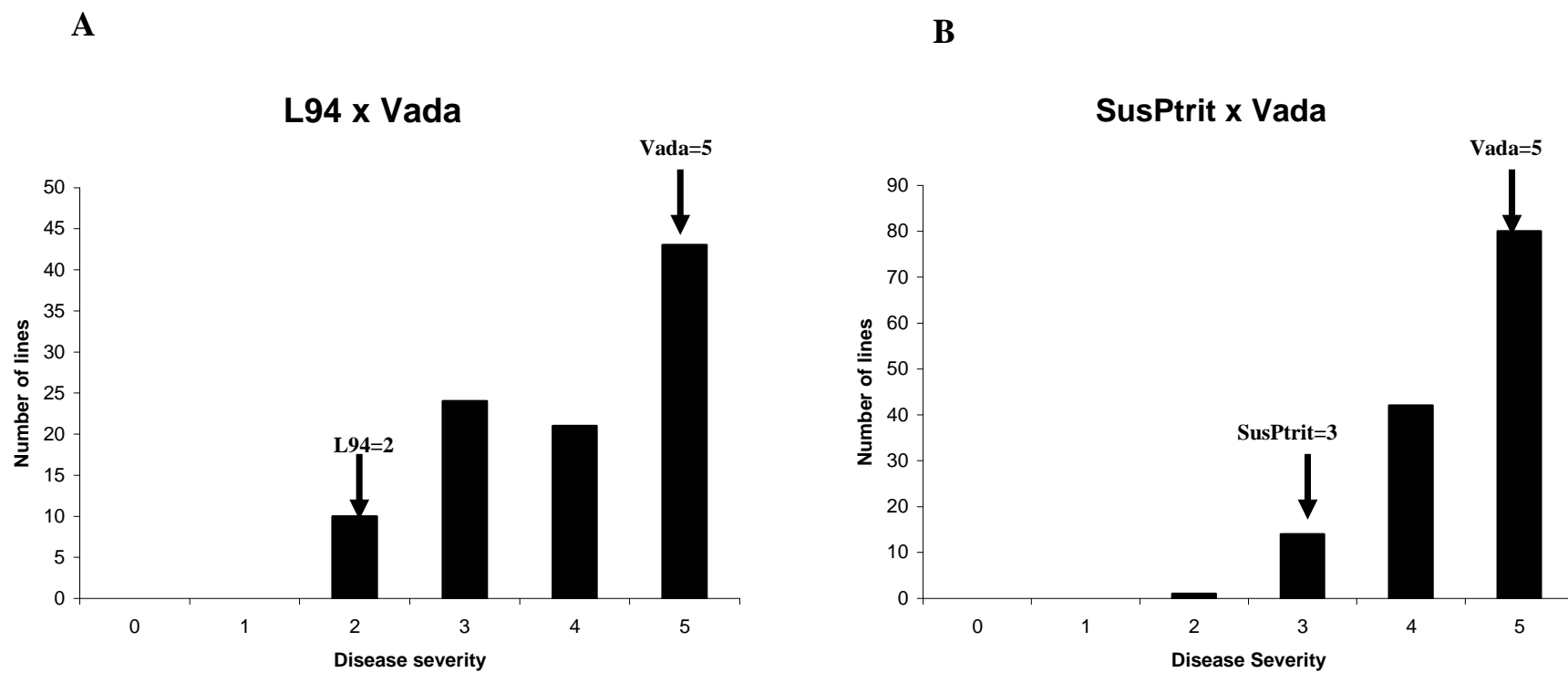


Figure 3: Histograms showing phenotypic frequency distribution of blast disease severity in RILs derived from **A-** L94 x Vada (LnVa), **B-** Vada x SusPtrit (VaSu) inoculated with a *tritici* isolate of *M. oryzae* isolate (PY67.1). The disease severity score values of the parents are indicated by an arrow.

3.3 QTLs mapped at adult plant stage

The expected blast symptoms were observed on all of the RILs of LnVa mapping population. The symptoms were on the spikes ranging from light-coloured elliptic lesions of the external parts of the glumes but without spikelet death to severe disease resulting in death of the whole spike. The parents differed greatly with L94 exhibiting light to moderate disease severity resulting death of one to seven isolated spikelets (score 2) and Vada exhibited severe disease severity resulting in death of the whole spike (score 4) (fig 4). The disease severity scores on the adult plants exhibited an approximate continuous variation in the LnVa RILs population tested indicating quantitative inheritance of the resistance (Fig 5). Transgressive segregation toward the resistance phenotype was observed indicating resistance at adult plant stage was contributed by both parents. Two QTLs were mapped on chromosome 2H and 7H. The QTL mapped on chromosome 7H had the largest effect, with a LOD value of 3.2 explaining 16.6% of the phenotypic variation and the resistance allele was contributed by Vada. The lesser effect QTL mapped on chromosome 2H, had a LOD value of 3.1, it explained 13.5% of the phenotypic variation and the resistance allele was contributed by L94. An overview of the QTLs is given in table 3. These results indicated that different QTLs are involved in *M. oryzae* resistance at seedling and at adult stage in LnVa population.



L94

Vada

Figure 4: Response of L94 and Vada parents of the LnVa population to blast isolate PY67.1 at adult plant stage. L94 showed moderate infection type with death of one to seven isolated spikelets and Vada showed severe disease severity with death of the whole spike. Plants were inoculated at flowering stage and were scored two weeks after inoculation.

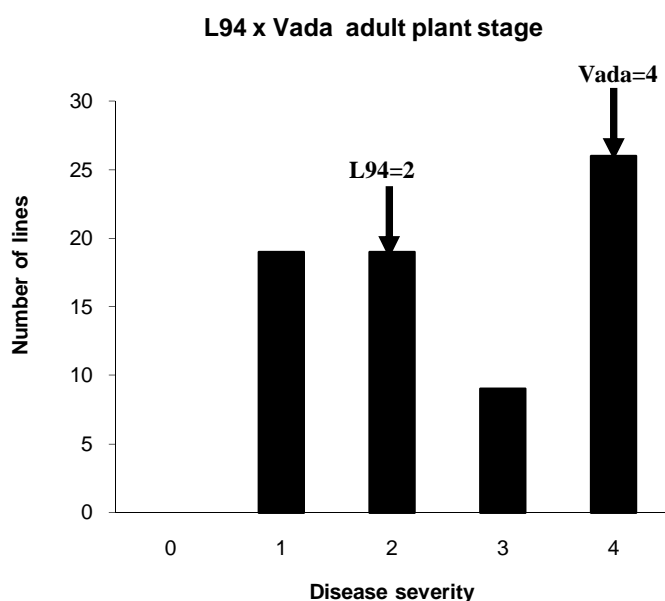


Figure 5: Histogram of the frequency distribution of blast disease severity scores at adult plant stage in 103 RILs of the LnVa mapping population inoculated with a *Triticum* isolate of *M. oryzae* (isolate PY67.1).

Table 3: Resistance Quantitative Loci (QTLs) to *Triticum* isolate PY67.1 of *M. oryzae*.

Population	Growth stage	Chromosome	Position of PM (cM)	Peak marker	LOD ^a	% exp ^b	Donor
LnVa	Seedling	7H	5.0	E33M55-508	17.14	54.6	L94
VaSu	Seedling	7H	5.6	E42M51-232	17.29	41.8	SusPtrit
LnVa	Adult	2H	83.9	Bmag0125	3.12	13.5	Vada
LnVa	Adult	7H	81.19	E39M61-372	3.23	16.6	L94

^a - Log of the likelihood of ratio at the peak marker position.

^b - percentage of the phenotypic variation explained by the QTL.

3.4 Marker-trait association

An overview of markers with their genome positions, correlations with blast resistance are shown in table 4. Only markers with a significant marker-trait correlation ($P \leq 0.01$) are shown. P-values and q-values for the correlations are also presented. Two markers were significantly correlated with blast resistance, and there were positioned on chromosome 5H and 7H. The most highly significant correlated marker for blast was the marker found on chromosome 5H at position 148.1 cM. Only one marker at position 148.1 cM on chromosome 5H had a false discovery rate less than 5% ($q \leq 0.05$). FDR is quite useful in genome wide studies where many markers are tested for association with a trait as it has high power in detecting marker-trait

association and it is stringent in declaring real marker trait associations (Kraakman et al., 2004).

In figure 6, association profiles where the p-value of the correlation of the markers and the trait is plotted as a function of map position of the markers on the chromosomes. This shows whether the associated marker stands out alone or a rise and fall in correlation occurs before and after the marker. A smooth rise and fall before an associated marker might point to a real marker-trait association. Only the chromosomes with significant marker-trait associations are shown. On chromosome 5H, a distinct peak appears at position 148.1, though two markers correlated with the trait appears before the peak causing an appearance of a ragged profile. On chromosome 7H a peak appears at position 133.2 cM, no markers were correlated with the trait before and after this peak resulting in the formation of a smooth rise and fall profile. Considering the fact that correlations found between the trait and markers located at less than 20cM to each other reflect presence of a QTL, we can suggest that two QTLs on chromosome 5H and 7H. Furthermore, the correlated marker at the peak of 7H coincided with one reported in literature in rice blast resistance in barley (Inukai et al 2006). The reported QTL by Inukai spanned from position 98-136.2 cM on chromosome 7H and the marker reported in this study on the same chromosome was on position 133.2cM just 3cM from the end of Inukai and other's QTL. The marker on chromosome 5H was 6.8cM from a QTL reported by Chen et al., 2003.

Table 4: Marker-trait correlation of AFLP markers with blast resistance, only markers with significant marker-trait correlations ($P \leq 0.01$) are shown. The position on the chromosome is given in cM and is based on barley integrated map of 2008 (Aghnoum et al., 2009). Significance of correlation is shown as a p-value and are indicated by **: $P < 0.01$ (significant); ***: $P < 0.001$ (highly significant). Correlation in bold had a false discovery rate of less than 5% ($q \leq 0.05$).

<i>Marker</i>	<i>Chromosome</i>	<i>Position (cM)</i>	<i>r</i>	<i>P-value</i>
E42M48-203	5H	148.1	0.3	***
E45M55-349	7H	133.2	0.27	**

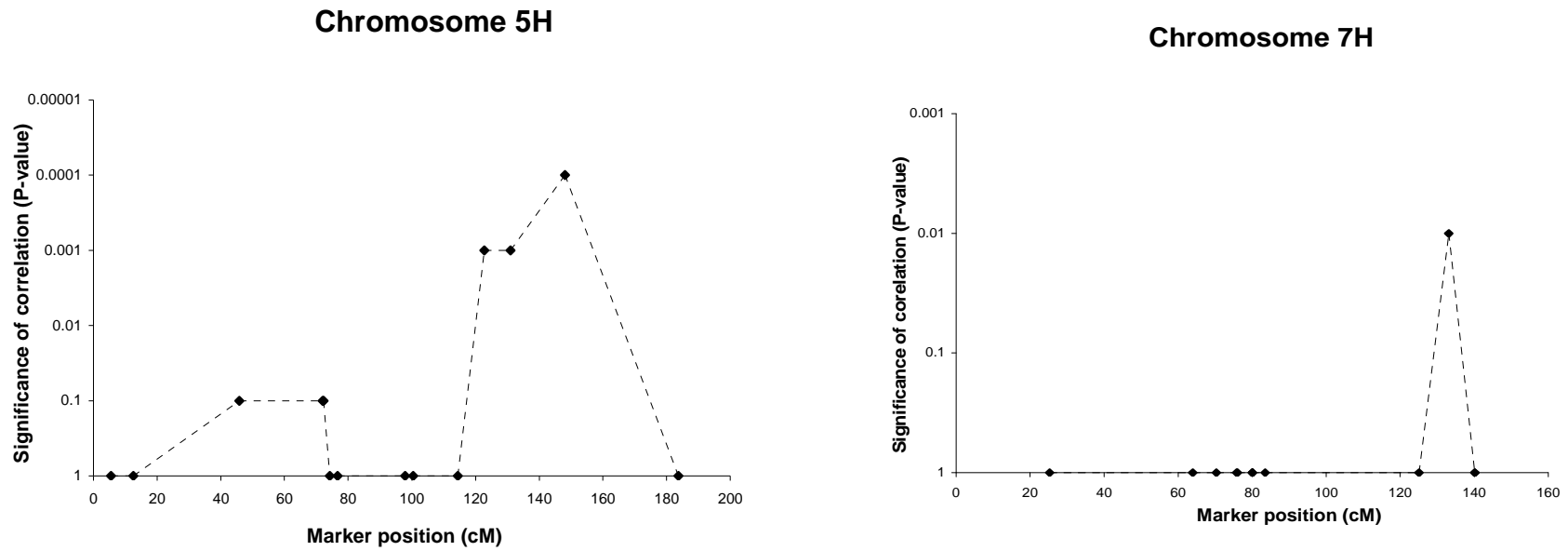


Figure 6: Association profiles showing p-values of correlation between markers and blast resistance plotted against chromosome positions of the markers. Only chromosomes with significant marker trait correlations are shown.

3.5 Genetic analysis of the complete resistance found in CGN02587

In order to investigate the inheritance of the complete resistance of CGN02587, an F₂ population derived from a cross between CGN02587 and Vada was inoculated with isolate PY67.1 and scored for disease severity. The frequency distribution of blast resistance showed a continuous distribution (Fig.7) and did not fall into discrete classes as expected in Mendelian inheritance. Thus we concluded that the inheritance of the resistance in CGN02587 is polygenic. DNA was isolated from the F₂ population for further analysis.

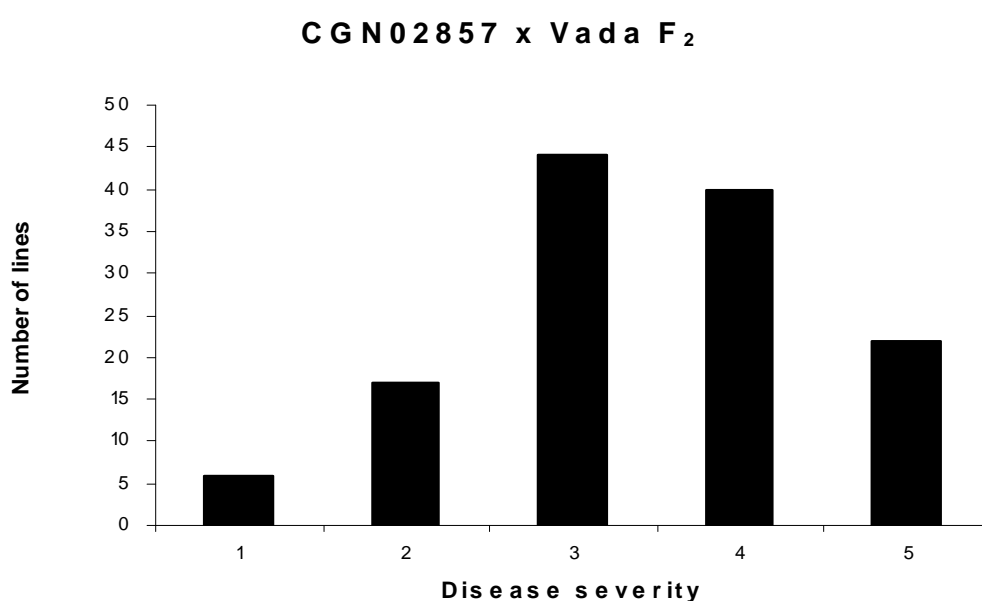


Figure 7: Histogram of the frequency distribution of blast severity in F₂ population derived from a cross between CGN02857 and Vada.

3.6 Histological analysis of the interaction of barley with *M. oryzae* at seedling stage

The interaction of CGN02587, L94 and Vada was evaluated at cellular level. Leaf samples were stained with Trypan blue, DAB or Uvitex at 14 hpi, 24 hpi and 48 hpi time intervals. Using bright field and fluorescence microscope, four different kinds of interaction phenotypes were distinguished and these reflected the success or failure of fungal invasion. The first phenotype was formation of appressorium with no staining or fluorescence of plant cells beneath it (**A**), the second was invasive hyphae formation with staining or autofluorescence of the attacked epidermal cells (**B**), the third was secondary hyphae formation with staining of regular shaped mesophyll cell (**C**)

and the last one was secondary hyphae spanning multiple cell associated with staining or strong autofluorescence of collapsed mesophyll cell (**D**). The first interaction phenotype represents the early stages of fungal development, the second shows epidermal hypersensitive reaction (HR), the third mesophyll HR to stop the fungus from spreading into the mesophyll cells and the fourth shows successful fungus spread into the mesophyll cells.

A quantitative evaluation of the leaf samples of each line per each time interval was done and the observed frequencies for the lines per each time point after inoculation are presented in Figure 9. There were no significant differences between the staining methods at the three time intervals. At 14 hpi two categories were observed, appressorium formation with no associated response (**A**) and invasive hyphae associated with death of the epidermal cell (**B**). The latter being only observed in CGN02587 and was significantly different to the other two lines. For category **A**, there were no significant differences between the lines.

At 24 hpi, most of the interaction sites evaluated showed a high number of unpenetrated cells as shown by high level of category A. However there was significant number of stained epidermal cells in CGN02587 and L94 compared to Vada, stained mesophyll cells were present in all the lines with L94 and Vada being significantly different from CGN02587. Only Vada had collapsed mesophyll cells. Another striking issue about Vada is that the fungus was able to establish secondary hyphae in the epidermal cells without the plant responding anyhow to arrest its spread.

At 48 hpi, accumulation of hydrogen peroxide which is an indicator of programmed cell death was monitored by DAB staining. There was a significant decrease in the level of category A over time with all the accessions having only 18-20% of interaction sites still in category A. In the rest of the interaction sites penetration of the fungus into the cells was observed. Hypersensitive reaction (HR) of epidermal cells was significantly high in CGN02587 than in L94 and was absent in Vada. L94 showed a high level of mesophyll cell HR which was significantly different from Vada only. In contrast high levels of stained collapsed mesophyll cells were observed in Vada in comparison to L94 and CGN02587, though L94 was significantly different from CGN02587. These results are consistent with the observed blast symptoms of the three lines, CGN02587 show only small brown pinpoint spots; this is because of the high epidermal and mesophyll cells HR which aborts pathogen invasion into the mesophyll cells thus no lesions visible to the naked eye. L94 shows an intermediate

response, with some epidermal and mesophyll stained cells as well as stained collapsed mesophyll cells, hence symptoms on its leaves appear as small necrotic spots and small brown lesion. On the contrast Vada had no epidermal cells staining, only staining of regular shaped and collapsed mesophyll cells. This shows the susceptibility of Vada as staining of collapsed mesophyll cell represent successful growth of the fungus in these cells. It is the successful spread of the fungus into the mesophyll cells which results in cellular disorganisation and cell collapse and this is a prerequisite for the formation of water soaked lesions visible to the naked eye. The contrasting responses of these three lines at the three time intervals are shown in figure 8.

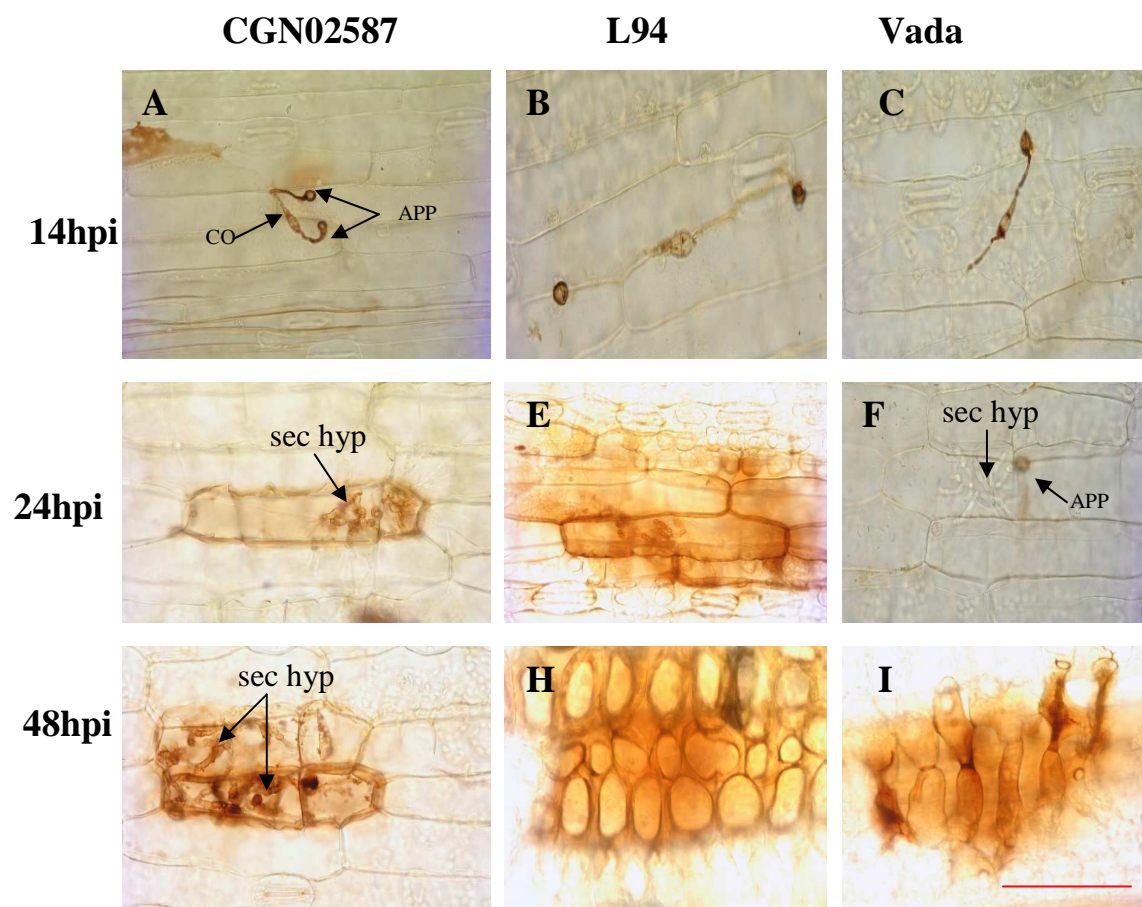


Figure 8: Microscopic interaction phenotypes of three barley lines (CGN02587, L94 and Vada) with *M. oryzae* observed under bright field microscope at three time intervals (14, 24 and 48hpi). **A, B** and **C**-Appressorium formation with no associated plant response, **D, E** and **G** -Invasive or secondary hyphae associated with death of the epidermal cell, **H** -Secondary hyphae associated with death of the mesophyll cells, **F** secondary hyphae with no associated plant response and **I**- Secondary hyphae growing in multiple cells associated with the collapse of adjacent mesophyll cells. **APP**- appressorium, **CO**- conidia, **sec hyp**- secondary hyphae, and scale bar=50µm

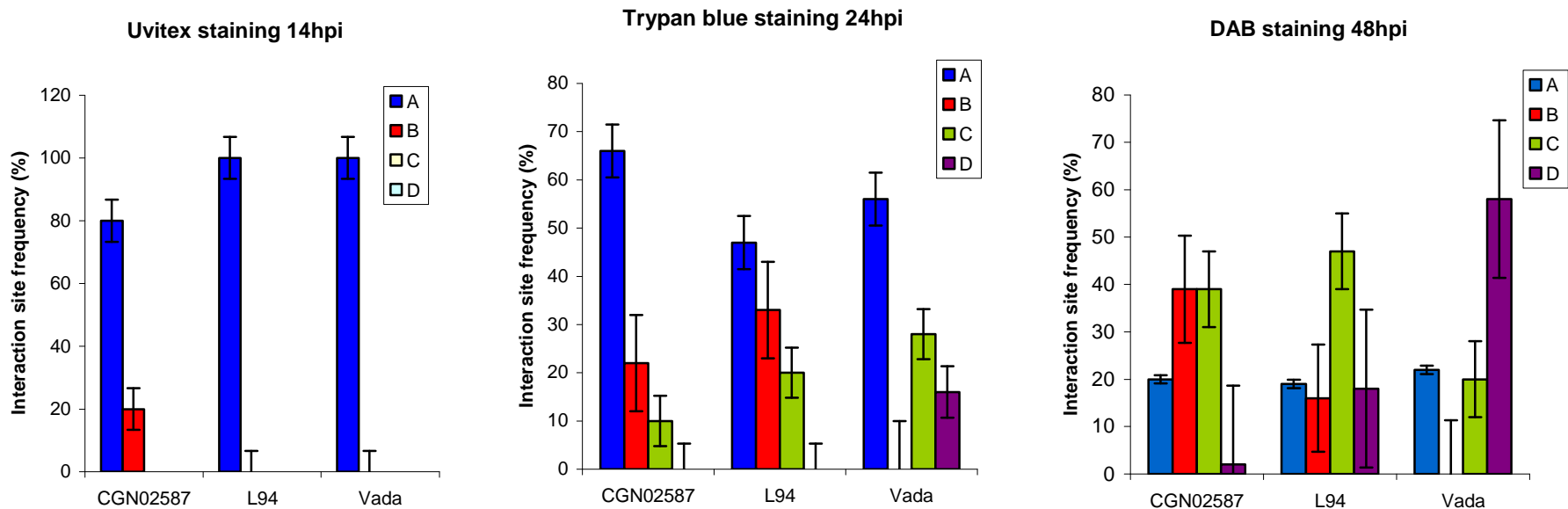


Figure 9: Microscopic evaluation of Uvitex, trypan blue and DAB staining of cellular interaction of three barley lines (CGN02587, L94 and Vada) inoculated with blast (*M. oryzae*) isolate PY67.1 at three time intervals (14hpi, 24hpi and 48hpi). For each staining method, one leaf segment was analysed per genotype, approximately 50 interaction sites were evaluated. The bars represent percentage mean of the two replicates for each barley line per scoring category and the error bars represents the standard error. Interaction sites were classified into four categories: **A**-Appressorium formation with no associated plant response, **B**-Invasive hyphae associated with staining or autofluorescence of the epidermal cell (epidermal HR), **C**-Secondary hyphae associated with staining or autofluorescence of regular shaped mesophyll cells (mesophyll HR) and **D**- Secondary hyphae growing in multiple cells associated with the collapse of adjacent mesophyll cells.

Chapter 4

Discussion

4.1 Host status of barley to *M. oryzae* isolates from wheat and rice

In this study it is shown that barley is a host to *M. oryzae* isolates from wheat and rice. However this is not a completely new phenomenon. Barley infection by *M. oryzae* isolates from wheat and rice have been reported already by Thomas 1940; Narita et al. 1956, Urashima et al. (1993), Greer and Webster 2001, Lima and minella (2003), Inukai et al., (2006), and Zellerhoff et al. (2006). It may be expected that pathotypes that are specific to wheat are compatible with barley as these two crops are closely related phylogenetically. However is interesting that a pathogen that is specific to rice also infect barley as rice and barley are from distantly related taxa though they shared some common ancestor millions of year ago (Kellogg 2001). Three plausible explanations arise to why barley is a host to these two pathotypes. The first one is host shifts may have occurred in the *Magnaporthe* pathogen. Host shift is when a pathogen starts infecting a new host that is not genetically far from its old host and this mainly happens in closely related crops that are co-cultivated, grown together in rotations or grown in close proximity (Stukenbrock and McDonalds 2008). Rice and barley, wheat and barley have a long history of being grown together in rotations in Thailand, Japan, Brazil and in Americas thus, it is most likely that the pathogen shifted to barley. Also, the pathogen's nature of high production capacity (can produce up to 6000 conidia per day up to fourteen days with multiple cycles) and the fact that the initial stages of infection are non specific; these could contribute to high level of infection on novel host species (Couch et al., 2005). However a conclusion can only be reached when evolutionary relationship of *M. oryzae* haplotypes from rice, wheat and barley is analysed. The second and third explanations could be that barley maybe an alternate host for the two pathotypes, and that it is a host that facilitates genetic recombination among different pathotypes in nature thus making barley a universal host plant to many other forms of blast (Couch et al., 2005). The latter has been previously reported in Italian ryegrass, tall fescue (Kato et al., 2000), and species of the *Aveneae* and *Festuceae* which are host to many pathotypes of *Magnaporthe* playing a role of facilitating recombination of different pathotypes in nature (Kato et al., 1980; 1983 in Kato et al., 2000).

Of the nineteen barley accessions tested in the isolate specificity experiment, six showed isolate specific resistance to the wheat isolates and eight to the rice blast isolates tested. This suggests that occurrence of different physiological races of wheat and rice blast pathogen which corresponds to the barley genotypes. Sato et al. (2001) showed that three barley lines (C651, I656 and I685) had isolate specific resistance to the rice blast isolates they had tested. However, proper differentiation of the virulence spectrum of the isolates using differential barley cultivars will further entail the different races in the *Tritici* and the *Oryzae* isolates of *Magnaporthe oryzae*.

4.2 Genetics of blast resistance in barley

Resistance of barley to blast was investigated in this study in three ways; (a) QTL mapping in two RILs populations, (b) association mapping in a collection of spring barley cultivars and (c) studying of the complete resistance of CGN02587 in a F₂ segregating population of a cross between CGN02587 and Vada. For QTL mapping, it is demonstrated that one QTL confer partial resistance to a *Tritici* isolate of blast in barley at seedling stage in two RILs populations LnVa and VaSu and that two QTLs are responsible for the same trait at adult stage in LnVa. To our knowledge these are the first QTLs for wheat blast resistance (*Triticum* pathotype of *M. oryzae*) in barley. It is interesting to note that at seedling stage, one QTL was responsible for the trait in both populations (LnVa and VaSu); this shows lack of diversity for QTLs for wheat blast resistance in these two populations. Since the resistant alleles of the QTL in LnVa and VaSu are contributed by L94 and SusPtrit respectively, and L94 was one of the lines used to develop SusPtrit (Atienza et al., 2004) it can be reasoned that blast resistance allele in SusPtrit are from L94. Further studies are however needed to make concrete conclusions.

The QTLs mapped at seedling stage were different from the QTLs mapped at adult stage none of them was effective at both growth stages, suggesting that these QTLs for blast resistance are growth stage dependent. Growth stage dependent QTLs have been reported in barley's interaction with other pathogen like powdery mildew (Aghnoum et al., 2010) and leaf rust (Qi et al. 1999). In comparison to the QTLs mapped for *M. oryzae* pathotype *oryzae* (rice blast) resistance in other barley populations (Inukai et al. 2006, Chen et al. 2003, Sato et al. 2001), none of the QTLs mapped in this study co-localised with the QTLs mapped for rice blast resistance in barley other studies. It is quite interesting that both the *Triticum* and the *Oryzae*

pathotypes are able to infect barley yet different regions of the barley genome are responsible for wheat and rice blast resistance. This further confirms that, though the *Triticum* and the *Oryzae* pathotypes are in the same lineage, they are true species genetically isolated from each other and hence, may illicit different resistance regions of the genome of the same plant specie. It will be however interesting to test this hypothesis on the same populations used in this blast study with *M. oryzae* isolates from rice.

In association mapping studies misinterpretation of marker-trait associations is a major source of error; all marker trait associations should be carefully checked to avoid this error. Three main criteria used in distinguishing real marker trait-associations from false ones are the significance of the marker-trait correlation, chromosomal LD profiles, and marker trait association and QTLs reported in other studies (Kraakman et al., 2006). However the latter had limitations in this as no QTLs have been so far reported for wheat blast resistance in barley, we considered those QTLs mapped for rice blast in barley. All these criteria were employed in this study to declare real marker trait associations. Two markers on chromosome 5H and 7H were associated with wheat blast resistance; it is interesting that the marker on chromosome 7H coincided with a QTL reported for rice blast resistance and this QTL maps to the same region as the powdery mildew resistance gene *Mlf*. This coincidence further confirms the association of blast resistance with powdery mildew resistance reported earlier by Jarosch et al 1999, Sato et al. 2001, Chen et al. 2003 and Inukai et al 2006. This may be attributed to the findings that monocots share certain pathways in resistance response thus genes underlying resistance QTLs or markers are commonly involved in defense response against pathogens (Chen et al., 2003).

The resistance of CGN02587 is probably quantitative as suggested by the lack of classical Mendelian segregation ratios and the normally distributed disease severity scores. According to the histological analysis discussed below, the resistance is hypersensitive based and according to the isolate specificity experiment (section 3.1) it is race non-specific as it was equally effective to all the pathogen isolate tested. Summing it all up, it can be suggested that CGN02587 shows a high level of partial resistance to wheat blast as it fits most of characteristics of this category. This however is inconclusive as the resistance has to be elucidated genetically at the DNA level. The segregating population can be genotyped and the loci regulating this resistance can be mapped. Bulk segregant analysis offers a rapid and cost effective

procedure for identifying markers in specific regions of a genome (Michelmore et al., 1991) and is ideal in analysis of the resistance of CGN02587 genetically.

4.3 Histological analysis of barley- *M. oryzae* interaction at seedling stage

Insights in to the infection process of *M. oryzae* on barley were gained by microscopic analyses of three barley accession which were fully resistant, partially resistant and susceptible to the pathogen. The infection process of *M. oryzae* isolate PY67.1 from wheat on barley resembled that which has been previously reported for different *M. oryzae* isolates from rice on barley (Jarosch et al., 2005; Zellerhoff et al., 2006, 2008). Germination of the spores and appressorium formation occurred within 14hpi, in all the three barley accessions analysed, this fits in the previously published results of different isolates of *M. oryzae* interaction with wheat (Tufan et al. 2009), rice (Fillipi, 2004) and barley (Zellerhoff et al., 2006) that germination and appressorium formation of *M. oryzae* occur between 2-20 hpi. This is mainly because appressorium formation of *M. oryzae* is generally the same and appears to be independent of the host specie (Howard and Valent 1996). Penetration occurred between 24 and 48 hpi and this was in contrast to an interval of 22 -28 hpi previously reported for other pathotypes of the fungus with different hosts suggesting that penetration takes longer in barley than in other hosts.

In CGN02857 the pathogen could successfully penetrate the epidermal cell, followed by establishment of the secondary hyphae in the epidermal cells and this resulted in high rate of staining or autofluorescence of epidermal and regular shaped mesophyll cells. Time course study showed that there were no collapsed mesophyll cells in CGNO2587 indicating that the pathogen could not successfully spread to the mesophyll cell but was halted in the epidermis. Thus the resistance of CGN02587 was based on epidermal and mesophyll HR. Similarly, this effective defense was shown to be crucial for R gene triggered response in rice-*Magnaporthe* interactions (Koga 1994), and for non host barley-*Magnaporthe* interactions (Zellerhoff et al., 2006). Furthermore it was reported for goose grass, weeping love grass and rice with intermediate compatibility to different strains of *Magnaporthe* (Heath et al., 1990). However, the above mentioned result is contrary to the previously published conclusion of Jarosch et al., 1999, 2005 that host resistance of barley to adapted *M. oryzae* isolates correlates to the formation of papillae at the sites were penetration failed and that epidermal HR in barley- *Magnaporthe* interaction does not arrest

fungal growth but only slows down its spread into the mesophyll cells. From this study, it is demonstrated that these conclusions should be revised as no papillae was formed and fungus spread was arrested in the epidermis by epidermal and mesophyll HR in the completely resistant accession CGN02587.

In contrast, in the interaction of L94 with *Magnaporthe*, the epidermal HR in L94 could not completely arrest the fungus from spreading into the mesophyll cells, the invaded mesophyll cell collapsed and underwent an HR like cell death. In this case it can be reasoned that the HR cell death in the mesophyll advocated for the development of the fungus. This is typical of the *M. oryzae* fungus which is a facultative biotroph and exhibits a necrotrophic habit at later stages of infection (Jarosch et al., 2005, Zellerhoff et al., 2006). This is also applicable for Vada as HR cell death in the mesophyll cells was the only significant responses observed and yet it was susceptible to wheat blast. These results resemble those reported for the interaction of, barley (Zellerhoff et al., 2006), wheat (Tufan et al., 2009), Lovegrass (*Eragrostis*) and rice (Heath et al., 1990) with *Magnaporthe* in highly compatible interactions.

4.4 Conclusions and recommendations

In conclusion, this research demonstrated that indeed barley is a host of blast *triticum* and the *oryzae* pathotypes of (*M. oryzae*) though. It went on further to identify QTLs and markers that confer resistance to the *triticum* pathotype of (*M. oryzae*). As this research was done under controlled conditions it is not known whether at the field conditions barley infection could occur with these isolates, whether the infection can originate from rice or wheat infections and more- still whether the same QTLs and markers can be responsible for the resistance. This study also added new insight of *barley-Magnaporthe* interactions at cellular level that host resistance of barley to adapted *M. oryzae* isolates is not necessarily correlated to the formation of papillae at the sites where penetration failed and that epidermal HR may succeed in arresting fungal growth into the mesophyll cells. The results of this study are however preliminary as agronomic utility of these QTLs, markers and mechanism of resistance still merits further investigations for it to be realized.

I highly recommend that in future studies other components of wheat blast resistance at seedling stage should be incorporated in the study. Components like infection frequency, lesion growth rate, lesion colour and lesion size (Tabayashi et al.,

2002). These have been described as important in rice-*Magnaporthe* (Castano et al. 1989) and in wheat-*Magnaporthe* interactions and can be useful in dissection *Magnaporthe* resistance in barley. In addition precision in terms of disease scoring scale, scoring method and the people doing the actual scoring is highly recommended.

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