GENETIC AND MOLECULAR ANALYSES OF RESISTANCE TO RUST DISEASES IN BARLEY

by

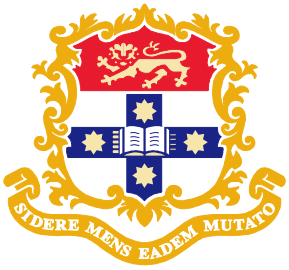
PRASHANT G. GOLEGAONKAR

M.Sc. (Agriculture), M.Sc. (Horticulture)

A thesis submitted in fulfilment

of the requirements for the degree of

DOCTOR OF PHILOSOPHY



THE UNIVERSITY OF SYDNEY
PLANT BREEDING INSTITUTE COBBITTY

NOVEMBER 2007

Statement of authorship

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and to the best of my knowledge, it contains no material previously published by any other person, except where due references are made in the text.

_____

Prashant G. Golegaonkar

ACKNOWLEDGEMENT

I take this opportunity to express my indebtedness to my supervisor Prof. Park, R.F., Director of Cereal Rust Research, Plant Breeding Institute of University of Sydney, Camden for his keen interest, most valuable and inspiring guidance, constant criticism throughout the research work and preparation of this manuscript.

I owe my profound thanks to my assistant supervisors, Assoc Prof. Wellings, C. R. and Dr. Singh, D. for their unfailing help and making enlightening suggestions during the course of the research. I am thankful to Dr. Karaoglu, H. for helping me to conduct molecular research. I am also thankful to Mr. Williams, M., Mr. Kavanagh, P., Mr. Kandel, K. and Ms. Wildner, B. for valuable technical assistance and Mr. Bell, J. A. for providing research facilities and necessary support for the research.

The financial support provided through industrial research scholarship by Grains Research and Development Corporation (GRDC) to conduct the research is gratefully acknowledged.

My special thanks to Emeritus Prof. McIntosh, R. A. for giving valuable time to discuss results and suggesting valuable information during the study.

I express my cordial thanks to my college mates Aderajew, Ali, Baljit, Omid, Natalia, Brett, Adrian, Jianping and a special friend, Tracy (Hiu Tong) Wong for their help during my studies.

I cannot eschew to express my wholehearted sense of reverence to my beloved wife and family members who were instrumental in providing this opportunity for building up my educational carrier, encouragement and inspiration throughout the study. At the last but not the least, I would like to be in awe of my daughter, Sanika for refreshing me from my tiresome research work with her sweet smile and affectionate cuddle.

"The reason of my liking to keep the marks for cleanness on plants, which have afterwards become rusty, is that I look upon lateness in becoming affected by it, as a measure of resistance to the parasite; and because I consider that individual plants may transmit to their progeny the valuable quality of resisting the parasite until a late stage of their lives, when it has less time for injuring the grain."

Farrer, W. J. (1898). The making and improvement of wheats for Australian conditions. Agr. Gas. N.S. W. **9**: 131-168.

Dedicated to

All scientists who contributed to the current understanding of rust resistance in cereals

Summary

The responses of 92 barley genotypes to selected *P. hordei* pathotypes was assessed in greenhouse tests at seedling growth stages and in the field at adult plant growth stages to determine known or unknown resistances. On the basis of multipathotype tests, 35 genotypes were postulated to carry *Rph2*, *Rph4*, *Rph5*, *Rph12*, *RphCantala* alone or combinations of *Rph2* + *Rph4* and *Rph1* + *Rph2*, whereas 52 genotypes lacked detectable seedling resistance to *P. hordei*. Five genotypes carried seedling resistance that was effective to all pathotypes tested, of which four were believed to carry uncharacterised resistance based on pedigree information. Field tests at adult plant growth stages indicated that while 28 genotypes were susceptible, 57 carried uncharacterised APR to *P. hordei*. Pedigree analysis indicated that APR in the test genotypes could have been derived from three different sources. The resistant responses of seven cultivars at adult plant growth stages were believed to be due to the presence of seedling resistance effective against the field pathotypes.

Genetic studies conducted on 10 barley genotypes suggested that 'Vada', 'Nagrad', 'Gilbert', 'Ulandra (NT)' and 'WI3407' each carry one gene providing adult plant resistance to *P. hordei*. Genotypes 'Patty', 'Pompadour' 'Athos', 'Dash' and 'RAH1995' showed digenic inheritance of APR at one field site and monogenic inheritance at a second. One of the genes identified in each of these cultivars provided high levels of APR and was effective at both field sites. The second APR gene was effective only at one field site, and it conferred low levels of APR. Tests of allelism between resistant genotypes confirmed a common APR gene in all genotypes with the exception of 'WI3407', which based on pedigree information was genetically distinct from the gene common in 'Vada', 'Nagrad', 'Patty', 'RAH1995' and 'Pompadour'.

An incompletely dominant gene, *Rph14*, identified previously in an accession of *Hordeum vulgare* confers resistance to all known pathotypes of *P. hordei* in Australia. The inheritance of *Rph14* was confirmed using 146 and 106 F3 lines derived from the crosses 'Baudin'/ 'PI 584760' (*Rph14*) and 'Ricardo'/'PI 584760' (*Rph14*), respectively. Bulk segregant analysis on DNA from the parental genotypes and resistant and susceptible DNA bulks from F_3 lines using diversity array technology (DArT) markers located *Rph14* to the short arm of chromosome 2H.

Polymerase chain reaction (PCR) based marker analysis identified a single simple sequence repeat (SSR) marker, Bmag692, linked closely to *Rph14* at a map distance of 2.1 and 3.8 cM in the populations 'Baudin'/ 'PI 584760'and 'Ricardo'/'PI 584760', respectively.

Seedlings of 62 Australian and two exotic barley cultivars were assessed for resistance to a variant of *Puccinia striiformis*, referred to as *BGYR*, which causes stripe rust on several wild *Hordeum* species and some genotypes of cultivated barley. With the exception of six Australian barley cultivars and an exotic cultivar, all displayed resistance to the pathogen. Genetic analyses of six Australian barley cultivars and the Algerian barley 'Sahara 3771', suggested that they carried either one or two major seedling resistance genes to the pathogen. A single recessive seedling resistance gene, *Bgyr1*, identified in 'Sahara 3771' was located on the long arm of chromosome 7H and flanked by restriction fragment length polymorphism (RFLP) markers wg420 and cdo347 at genetic distances of 12.8 and 21.9 cM, respectively. Mapping resistance to *BGYR* at adult plant growth stages using a doubled haploid population derived from the cross 'Clipper'/'Sahara 3771' identified two major QTLs on the long arms of chromosomes 3H and 7H that explained 26 and 18% of total phenotypic variation, respectively. The QTL located on chromosome 7HL corresponded to the seedling resistance gene *Bgyr1*. The second QTL was concluded to correspond to a single adult plant resistance gene designated *Bgyr2*, originating from cultivar 'Clipper'.

Contents

List of Tables

List of Figures

List of Plates

CHAPTER I

Introduction

1.1 Taxonomy and origin

The genus *Hordeum* belongs to the *Triticeae* tribe of the *Poaceae*, the largest family within the monocotyledonous plants, and comprises 32 species and altogether 45 taxa of which *H. vulgare* ssp. *vulgare* is the only species that has undergone domestication (Von Bothmer *et al*., 1995). Barley is considered to be one of the founding species of modern agriculture. It was domesticated about 10,000 years ago from the wild progenitor *H. vulgare* spp. *spontaneum*, most probably in the western part of the Fertile Crescent (Badr *et al*., 2000). Cultivated barley (*Hordeum vulgare*) is a self pollinating diploid with a chromosome number of 2n=14. Barley cultivars are mainly classified on the basis of vernalisation requirement (spring or winter type), spike morphology (six row, two row and some intermediate forms), end use (malting or feed) and presence of hull on seed (hulless or hulled).

1.2 Cultivation and use of barley

Barley is an important cereal crop, ranking fourth in world food production next to maize, rice and wheat. In 2003, total global barley production was 139 million metric tonnes on an area of 55 million hectares (FAO, 2004). Barley is also one of the hardiest cereal crops, growing in a wide variety of environments that include extremes of latitude and altitude where other crops are not adapted (Harlan, 1976). Because of its greater tolerance to soil salinity, barley can be grown in areas that are unsuitable for wheat (Harlan, 1995). The major barley production areas are Europe, the Mediterranean fringe of North Africa, Ethiopia, The Near East, Russia, China, India, Canada, USA and Australia. Barley is an important source of animal feed and brewing malts and is important for human consumption.

The Australian barley industry contributes a small but important part of the national economy and farm sector. It is the second most important cereal crop after wheat and is grown in all cereal growing regions. South Australia is the largest barley producing state, followed by Western Australia, New South Wales and Victoria. The majority of barley produced in Australia is exported. Australian exports

contribute approximately 18% of the total world trade of barley. The remainder is used domestically for feeding animals or for malting and brewing.

1.3 Barley breeding

Global barley production has increased steadily over the last 40 years (Langridge and Barr, 2003). Though fertilisers and pesticides have contributed increased yield and production, most of these increases have come from improvements in genetic make up brought about by breeding. Recent developments in biotechnological methods have provided important tools to supplement conventional breeding methods (Langridge and Barr, 2003). The most common barley breeding objectives are to produce cultivars that are early maturing, dwarf to semi-dwarf in stature, high yielding, resistant to biotic and abiotic stresses, and have good malting quality.

1.3.1 Disease resistance

Diseases can seriously reduce grain quality and final yield, resulting in a lower financial return to growers. In Australia, at least 23 diseases are reported to damage barley, the major ones being leaf scald, leaf rust and the spot form and the net form of net blotch (Williams, 2003). Barley leaf rust (caused by the fungus *Puccinia hordei*) occurs in all barley growing regions of Australia and has often reached epidemic levels, causing significant yield losses (Park, 2003). The deployment and development of disease resistant cultivars is the preferred method of disease control as it avoids potential harmful effects of chemicals on environment. To date, 19 loci conferring seedling resistance to *P. hordei* have been characterised in barley, however, very few remain effective against current pathotypes of *P. hordei*. Recent studies have indicated that most popular Australian barley cultivars lack effective resistance against *P. hordei* (Park, 2003; Cotterill *et al*., 1995). Through marker assisted selection, combining genes that are still effective could facilitate their incorporation into new cultivars to achieve longer lasting resistance. In addition to seedling resistance genes, some barley cultivars are known to carry slow rusting that provides adequate resistance at adult plant growth stages (Park, unpublished; Cotterill and Rees, 1993). Adult plant resistance (APR) to rust is known to be an important component of some sources of durable resistance in wheat (Singh *et al*., 2001). Finding and characterising such sources of resistance to *P. hordei* in barley could facilitate their utilisation in breeding programmes.

A new form of stripe rust (caused by *P. striiformis*), virulent to some barley cultivars, was first identified in 1998 on barley grass in Australia (Wellings *et al*., 2000a). Given the widespread occurrence of barley grass in barley growing regions of Australia, understanding host-pathogen interactions in this new pathosystem could be useful to understand the potential threat of the disease to barley cultivation. Though most commercial malting grade barley cultivars are resistant to this pathogen, knowledge of the number of genes for resistance, their mode of inheritance and relationship with resistance to the barley stripe rust pathogen (*P. striiformis* f. sp. *hordei*) will be useful in future barley breeding efforts.

1.4 Research aims

The broad objectives of this study are:

1. To assess a set of European barley accessions at seedling and adult plant growth stages for the presence of resistance to *P. hordei*
2. To conduct studies on the inheritance of APR in 10 barley genotypes and to determine the genetic relationship between the APR genes present in these cultivars by tests of allelism
3. To determine the chromosomal location of seedling resistance gene *Rph14* and to find a closely linked molecular marker for use in marker assisted selection
4. To conduct genetic and molecular analysis of resistance to *BGYR* in selected barley cultivars

CHAPTER II

Review of literature

2.1 General introduction

Rusts are the most destructive diseases of cereals and have caused widespread losses in wheat, oat, barley and related crops. The earliest records of a rust disease occur in the Bible and in Greek and Roman literature, going back to 500 BC. These records include details of a ceremony to mollify Robigus, the Corn God, to avoid crop failure due to rust disease. Evidence of the presence of *Puccinia graminis* on wheat lemma fragments dated to 1400-1200 B.C. were reported by Kislev (1982). These earliest records show that the development of cereal rusts have paralleled the domestication of cereal crops.

The cause of rust diseases remained a mystery until the mid 17th Century. According to Schafer *et al.* (1984), Fontana was the first person to discover that fungi are the causal organisms of rust. It was only in the later part of the 19th Century that the rust fungi were recognised as distinct and categorised into separate classes. More than 3000 species of rust fungi have been described (Laundon, 1973). The rust fungi are phyto-pathogenic microfungi that comprise the order *Uredinales* of the phylum *Basidiomycota*. They are referred to as rust fungi because of the characteristic rust-coloured spores produced on plants. The complex life cycles, obligate parasitism and potential for crop losses make rust fungi of great biological interest (Park, 2000).

In cereal crops, there are three types of rust diseases i.e. leaf rust, stem rust and stripe rust. The importance of each disease in each crop depends on geographic location and environmental conditions prevailing in the area. Primary infection can be caused by wind-borne urediniospores or aeciospores. The disease develops fast when free moisture occurs due to rain or dew. However, the optimum temperature requirement for the rapid development of disease differs for each rust pathogen species. The stripe rust pathogen requires a temperature range of 10-20^{0}C, while the pathogens that cause leaf rust and stem rust require near 20^{o}C and about 20^{o}C and more, respectively. The symptoms associated with all three pathogens are different and can be easily categorised by visual observation. In the case of the leaf rust pathogens of wheat and barley, infection sites are found primarily on the upper surfaces of leaves and leaf sheaths. Dark reddish brown pustules on both sides of leaves, stems and

spikes are the characteristic symptoms of stem rust. The pustules of stripe rust, which contain yellow to orange-yellow urediniospores, usually occur in narrow stripes on the leaves. Pustules can also be found on leaf sheaths, necks and glumes. Because of the characteristic colour of uredinia produced by the pathogens of leaf rust, stem rust and stripe rust, they are also referred to as brown rust, yellow rust and black rust, respectively in some countries. All three fungi produce dark black coloured telia when the host plant matures.

Many scientists have made significant efforts to classify rust fungi. Eriksson (1894) found that cereal rust fungal species are not homogeneous in their host ranges. He noted that isolates of rust that were morphologically indistinguishable differed in their abilities to infect different host species. This concept led to the classification of rust fungi into *taxa* within species that are designated *formae speciales* (singular *forma specialis*). However, there is some controversy over this classification. According to Anikster (1984), many scientists believe that rust organisms use wild grasses as a source of primary infection before passing onto cultivated cereals, or they attack only wild grasses. If the former concept is true, classification based on *formae speciales* is not valid. Host overlapping between *formae speciales* is also common. *Formae speciales* can also undergo asexual recombination to produce somatic hybrids (Watson and Luig, 1959; Luig and Watson, 1972). The *formae speciales* are in turn composed of races (physiological forms, pathotypes), which differ in their virulence for resistance genes carried by the primary host.

2.2 Rust diseases in barley

Barley is affected by three rust diseases (leaf rust, stripe rust and stem rust). Leaf rust is caused by *P. hordei*, stripe rust is caused by either *P. striiformis* f. sp. *hordei*, or less commonly, wheat stripe rust (caused by *P. striiformis* f. sp. *tritici*). In addition, a new variant of *P. striiformis*, commonly known as *BGYR* (barley grass stripe rust) is also known to infect barley in Australia (Wellings *et al.*, 2000a). Stem rust in barley can be caused by the form that infects wheat (*P. graminis* f. sp. *tritici*), the form that causes stem rust in cereal rye (*P. graminis* f. sp. *secalis*), or a form regarded to be a somatic hybrid between f. spp. *tritici* and *secalis* (Luig and Watson, 1972).

2.2.1 Leaf rust of barley

Leaf rust (caused by *P. hordei;* syn. *P. anomala*, *P. simplex*) is an economically important foliar disease of barley in most temperate regions of the world including Australasia, Europe, North America and South America (Clifford, 1985). The causal pathogen is widespread in all barley growing regions of Australia and has caused yield losses since at least 1927 (Park, 2003).

2.2.1.1 Life cycle of *P. hordei*

P. hordei Otth. is a macrocyclic, heteroecious rust pathogen. Uredinia and telia occur on wild and cultivated *Hordeum* spp., with aecia occurring on *Ornithogalum*, *Leopoldia* and *Dipcadi* spp. in the family *Liliaceae*. Tranzschel (1914) was the first to implicate *Ornithogalum* as an alternate host of *P. hordei*. This was later confirmed by several researchers (Anikster, 1982). The alternate host has an important role in permitting the sexual cycle and thereby potentially generating genetic variability (Anikster, 1982). The life cycle of *P. hordei* has been classified into five stages on the basis of production of different spore types. Among the five spore stages, the urediniospore stage is economically the most damaging. Each urediniospore has the potential to infect the same host plant or another host plant. Multiple cycles of infection, sporulation and re-infection can produce very destructive epidemics in barley fields within just weeks. Urediniospores are produced in uredinia from dikaryotic mycelium. The spores are detached and carried over a long distance by wind, rain and several other agents to start a new infection. Dispersal of rust spores over a long distance enables them to colonise new regions rapidly. For example, pathotype distribution studies on *P. hordei* in Australia demonstrated that a pathotype virulent on *Rph12* (*pt.* 4610 P+) was first detected in Tasmania in 1991. This pathotype was subsequently detected in all barley growing regions of eastern Australia in 1998 (Park, 2003).

Free moisture is essential for germination of urediniospores and penetration of the host. Germination takes place from 5^{o}C to 25^{o}C, with 10^{o}C to 20^{o}C being optimal (Simkin and Wheeler, 1974a). Several studies have examined affects of environmental conditions on the growth and development of *P. hordei* (Simkin and Wheeler, 1974b; Teng and Close, 1978). Under optimal conditions, sporulation begins 6-8 days after infection (Polly and Clarkson, 1978), but can take up to 60 days at 5^{o}C (Simkin and Wheeler, 1974a). The amount of sporulation was similar between

10-20^{o}C, but declined at 25^{o}C. Uredinial size, generation time and sporulation period were all reduced as uredinial density increased (Teng and Close, 1978). Teng and Close (1978) reported that host colonisation is restricted by temperature and increases to a maximum in the range of 5-25^{o}C. After completion of the host life cycle, volunteer plants and wild *Hordeum* spp. act as a "green bridge" for survival of urediniospores during the summer season. The green bridge is important in the continuation of the vegetative life cycle of the pathogen where the sexual cycle does not exist. Teng and Close (1980) found that urediniospores lost viability rapidly when exposed to sunlight during warm summer days in New Zealand, but in simulated cloudy weather, spores survived for up to 38 days.

When the host matures, the pathogen produces black coloured telia in which dark brown or black coloured, thick walled teliospores are formed. Each cell of a teliospore carries two nuclei, which undergo fusion (karyogamy) to produce a single diploid nucleus. In winter, the spores attached to stubble or straw can remain dormant until suitable conditions occur, or they may germinate immediately to produce basidia. Haploid unicellular basidiospores are produced on a basidium via meiosis and the mature basidiospores are ejected forcibly and carried away by air currents. If they reach a susceptible species of *Ornithogalum*, the basidiospores germinate and penetrate the leaf. Pycnia, which result from infection on young *Ornithogalum* leaves, contain two key elements for the sexual process. Pycniospores are produced in a sugary nectar within the pycnia and function as male gametes. They consist of little more than a nucleus to fertilise the receptive hypha of another pycnium of compatible mating type. The receptive hyphae function as the female gametes. The sugary nectar released by the pycnia helps spread the pycniospores. Insects are attracted to the nectar and often visit several pycnia in succession, fertilising them much as bees pollinate flowers. Self fertilisation is prevented in *P. hordei*, because only + mating type pycniospores can fuse with - mating type receptive hyphae, and vice versa. The structure that results from fertilisation between a pycniospore and a receptive hypha becomes a dikaryotic aecium, in which chains of aeciospores are produced. Aeciospores can differ in virulence against different host genotypes if sexual recombination occurs between two genetically different haploid pycniospores. Aeciospores are carried away by wind to infect wild or cultivated *Hordeum* spp., producing a dikaryotic mycelium on which urediniospores are produced. These urediniospores are then able to perpetuate the cycle (Fig. 2.1).

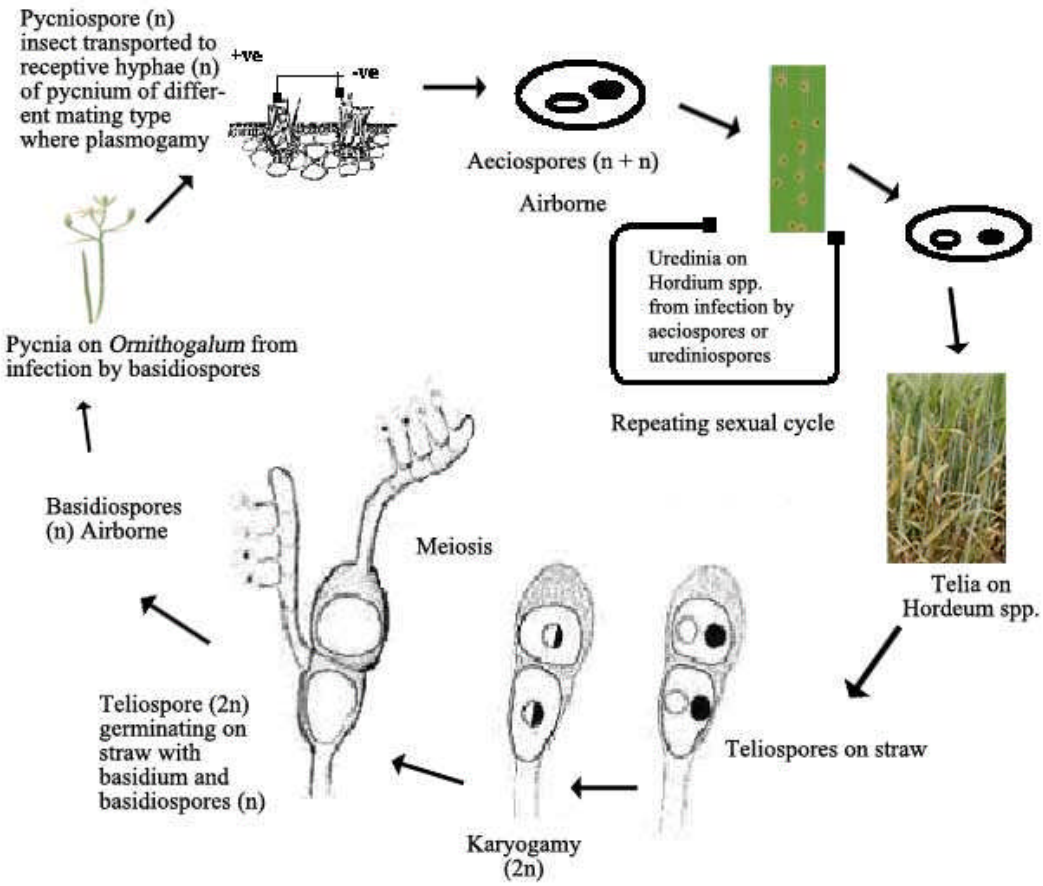


Figure 2.1. Life cycle of *P. hordei.* The cycle involves five spore stages; 0 – Pycniospores, I – Aeciospores, II – Urediniospores, III – Teliospores, IV – Basidiospores (designation of spore stages, see Park, 2000).

The alternate host has been reported as essential for the survival and generation of variability in the pathogen in Israel (Anikster *et al.*, 1976) and Greece (Critopoulos, 1956). However, it was regarded as unimportant in Central Europe and England because teliospore germination does not synchronise with the growth of *Ornithogalum* (Clifford, 1985). Manisterski (1989) reported that a dynamic virulence situation in Israel was due to genetic recombination in the gametophytic stage of *P. hordei* on *Ornithogalum,* which is widespread in Israel. Golan *et al.* (1978) isolated four cultures of *P. hordei* virulent on seedlings of barley cultivars carrying resistance genes *Rph1*, *Rph2*, *Rph2* + *Rph5*, *Rph2* + *Rph6*, *Rph3*, *Rph4*, and *Rph7* from the alternate hosts in Israel. These pathotypes had not been detected before in nature and hence were thought to originate from sexual recombination between existing pathotypes. The cultures remained stable as clones through 20 successive inoculation and isolation cycles.

In Australia, the alternate host *Ornithogalum umbellatum* occurs in localised parts of the Yorke Peninsula of South Australia. *P. hordei* is the only cereal rust that undergoes sexual recombination in Australia. Wallwork *et al.* (1992) showed that the environment of the York Peninsula is suitable for basidiospores of *P. hordei* to germinate and infect the alternate host and to produce large quantities of aeciospores. The aeciospores collected from naturally infected *O. umbellatum* were used to infect barley plants and seven separate single uredinial isolates yielded five different pathotypes.

2.2.2 Economic importance

Losses due to leaf rust are not reported to be widespread or to occur on a regular basis but the disease is important locally, especially in the cool temperature regions of barley cultivation. Potential yield losses in the range of 10 to 62% were reported in Australia (Waterhouse, 1927; Dill-Macky *et al.*, 1989; Cotterill *et al.*, 1992), Europe (King and Polley, 1976; Clifford, 1985), New Zealand (Teng and Close, 1978; Arnst *et al.*, 1979; Lim and Gaunt, 1986; Wright and Gaunt, 1992), North America (Newton *et al.*, 1945; Levine and Cherewick, 1956; Reinhold and Sharp, 1982; Mathre, 1982; Griffey *et al.*, 1994), the Netherlands (Wilten, 1953) and the United Kingdom (Johnson, 1970; Jenkins *et al.*, 1972; Melville *et al.*, 1976; King, 1977).

Yield losses occur mainly because of a reduction in seed size. Leaf rust affects photosynthesis, respiration and the transport of nutrients, resulting in a general debilitation. The shriveling of seeds not only affects total yield but also affects malting quality by reducing protein content.

In Australia, the first leaf rust epidemics in barley crops were reported in New South Wales in the 1920s (Waterhouse, 1927). Thereafter, there was little documentation of the occurrence of leaf rust till the 1980s, when epidemics occurred in Queensland (1978, 1983, 1984 and 1988), South Australia (1988), and in northern NSW and Tasmania (1990) (Cotterill *et al.*, 1992). These epidemics were attributed to intensified growing of barley, early and extended planting of crops and the use of susceptible cultivars (Cotterill *et al.*, 1992).

2.3 Pathotypes of *P. hordei*

During a study of *P. graminis* f. sp. *tritici* (*Pgt*), Stakman and Piemeisel (1917) found that two isolates differed in their abilities to infect two cultivars of wheat. These

studies became the basis to classify the rust fungi into different pathotypes. The virulence/avirulence pattern of a culture is determined by inoculating a selected group of host plants of differing genotype (known as a differential set) for rust resistance. Stakman *et al.* (1962) used 12 host cultivars to differentiate pathotypes of *Pgt*. The differential set is of great practical use in identifying existing and new pathotypes and in determining their distributions. Owing to the possible threat of barley leaf rust in different regions, intensive studies had been conducted to determine existing pathotypes and the effectiveness of resistance gene(s). Levine and Cherewick (1952) were the first to investigate the pathogenic specialisation of *P. hordei* on an international scale using isolates from North America, Europe and Australia. They differentiated 52 pathotypes of *P. hordei* based on nine differential varieties of barley. Most previous studies were conducted using local sets of differential genotypes. This practice hampered international efforts to exchange data regarding the distribution of pathotypes world-wide and resistance genes that were effective against them. To avoid this confusion, Clifford (1977) suggested two components to the differential series: 1) A standard set of International differential genotypes for comparative studies of virulence gene frequencies and associations on a global basis. 2) Regional sets of supplementary differentials relevant to the breeding and research interests of individual workers. This system facilitated the exchange of data regarding the distribution of pathotypes around the world. With the advancement of molecular markers, it is now possible to study genetic diversity among the different rust pathogens and within different pathotypes of a pathogen (Keiper *et al.*, 2003). These studies are fundamental for understanding the mechanisms generating genetic variation, host-pathogen co-evolution and ultimately the management of the disease (Aradhya *et al.*, 2001).

In Australia, studies of pathogenic specialisation were conducted regularly to determine the virulence avirulence of *P. hordei* (Waterhouse, 1927, 1952; Watson and Butler, 1947; Luig, 1985; Cotterill *et al.*, 1995; Park, 2003). These studies were important in understanding changes in the pathogen population and in identifying what genes are effective. The early work by Waterhouse (1927, 1952), Watson and Butler (1947) and Luig (1985) showed that two to three pathotypes with virulence on *Rph2*, *Rph4* and *Rph8* were present in Australia. Cotterill *et al.* (1995) identified 11 distinct pathotypes with combinations of virulence for *Rph1*, *Rph2*, *Rph4*, *Rph5*, *Rph6*, *Rph8*, *Rph9* and *Rph12* from 154 disease samples collected from 1966 to 1990. Park (2003) reported annual pathogenicity data and pathotype distribution of *P. hordei* in Australia

from 1992 to 2001. Most of the samples originated from southern NSW, Victoria and Tasmania. The data obtained revealed a significant shift in the composition of populations across four cereal growing regions of Australia with virulence for resistance gene *Rph12*. Pathotype 4610P+, virulent on *Rph12,* was first detected in Tasmania in 1991. This pathotype was subsequently detected in all regions except WA. A further seven pathotypes virulent on *Rph12* were identified after the initial detection of *pt.* 4610P+. Two pathotypes virulent on *Rph12* were detected in WA in 1997 and 2001 (viz. 5610P+ and 5453P-). The increase in virulence for *Rph12* in all cereal growing regions was believed to be due to the cultivation of barley cultivar with this gene. To date, pathotypes virulent on *Rph3*, *Rph7*, *Rph11*, *Rph14*, *Rph15* and *Rph18* have not been detected in Australia (Park, 2003). Based on the reaction of the Australian differential genotype set, 23 distinct pathotypes were identified from the isolates collected during the 1992 to 2002 annual surveys conducted by Plant Breeding Institute (PBI) and they were preserved in liquid nitrogen (Table 2.1).

2.3.1 Pathotype nomenclature

Stakman and Levine (1922) were the first to classify different pathotypes of *P. graminis* f. sp. *tritici* on the basis of pathogenicity on host genotypes. The system they developed was used by many scientists but has been largely discarded because there was no provision for the inclusion of new differential hosts, necessary because of changes in the distribution and virulence of new pathogens. In this context, open ended nomenclature systems have been found to be more useful. Gilmour (1973) suggested an octal/binary system to designate pathotypes. Clifford (1992) proposed to adopt this system for international use because it was informative, simple, logical, short and flexible, and has provision to incorporate new genotypes. The use of this system will enable researchers to understand the global distribution of *P. hordei* pathotypes.

To obtain a unique octal notation, differential cultivars carrying resistance genes are assigned a fixed linear order and grouped into sets of three. A binary number is initially assigned to each differential genotype where a resistant reaction = 0 and a susceptible reaction = 1. Based on binary numbers, binary triplet numbers are obtained for each set of three differential genotypes. The corresponding binary triplets can then be assigned their corresponding octal number (Table 2.2).

Table 2.1. *P. hordei* pathotypes in Australia and their virulence/avirulence pattern.

Pathotype [a]	**Virulence/avirulence formula on *Rph* gene/s**
20 P^+	*Rph5*, *Rph19*/ *Rph1*, *Rph2*, *Rph3*, *Rph4*, *Rph6*, *Rph7*, *Rph8*, *Rph9*, *Rph10*, *Rph11*, *Rph12*
200 P^+	*Rph8*, *Rph19*/ *Rph1*, *Rph2*, *Rph3*, *Rph4*, *Rph5*, *Rph6*, *Rph7*, *Rph9*, *Rph10*, *Rph11*, *Rph12*
200 P^-	*Rph8*/ *Rph1*, *Rph2*, *Rph3*, *Rph4*, *Rph5*, *Rph6*, *Rph7*, *Rph9*, *Rph10*, *Rph11*, *Rph12*, *Rph19*
201 P^+	*Rph1*, *Rph8*, *Rph19*/ *Rph2*, *Rph3*, *Rph4*, *Rph5*, *Rph6*, *Rph7*, *Rph9*, *Rph10*, *Rph11*, *Rph12*, *Rph19*
210 P^{+**}	*Rph4*, *Rph8*, *Rph19*/ *Rph1*, *Rph2*, *Rph3*, *Rph5*, *Rph6*, *Rph7*, *Rph9*, *Rph10*, *Rph11*, *Rph12*,*Rph13*
211 P^+	*Rph1*, *Rph4*, *Rph8*, *Rph19*/ *Rph2*, *Rph3*, *Rph5*, *Rph6*, *Rph7*, *Rph9*, *Rph10*, *Rph11*, *Rph12*
220 P^+	*Rph5*, *Rph8*, *Rph19*/ *Rph1*, *Rph2*, *Rph3*, *Rph4*, *Rph6*, *Rph7*, *Rph9*, *Rph10*, *Rph11*, *Rph12*
222 P^+	*Rph2*, *Rph5*, *Rph8*, *Rph19* / *Rph1*, *Rph3*, *Rph4*, *Rph6*, *Rph7*, *Rph9*, *Rph10*, *Rph11*, *Rph12*
230 P^+	*Rph4*, *Rph5*, *Rph8*, *Rph19*/ *Rph1*, *Rph2*, *Rph3*, *Rph6*, *Rph7*, *Rph9*, *Rph10*, *Rph11*, *Rph12*
232 P^+	*Rph2*, *Rph4*, *Rph5*, *Rph8*, *Rph19*/ *Rph1*, *Rph3*, *Rph6*, *Rph7*, *Rph9*, *Rph10*, *Rph11*, *Rph12*
242 P^+	*Rph2*, *Rph6*, *Rph8*, *Rph19*/ *Rph1*, *Rph3*, *Rph4*, *Rph5*, *Rph7*, *Rph9*, *Rph10*, *Rph11*, *Rph12*
243 P^+	*Rph1*, *Rph2*, *Rph6*, *Rph8*, *Rph19*/ *Rph3*, *Rph4*, *Rph5*, *Rph7*, *Rph9*, *Rph10*, *Rph11*, *Rph12*
253 P^-	*Rph1*, *Rph2*, *Rph4*, *Rph6*, *Rph8*/ *Rph3*, *Rph5*, *Rph7*, *Rph9*, *Rph10*, *Rph11*, *Rph12*, *Rph19*
262 P^+	*Rph1*, *Rph2*, *Rph4*, *Rph6*, *Rph8*/ *Rph3*, *Rph5*, *Rph7*, *Rph9*, *Rph10*, *Rph11*, *Rph12*, *Rph19*
272 P^+	*Rph1*, *Rph2*, *Rph4*, *Rph5*, *Rph6*, *Rph8*/ *Rph3*, *Rph7*, *Rph9*, *Rph10*, *Rph11*, *Rph12*, *Rph19*
4610 P^+ [b]	*Rph4*, *Rph8*, *Rph9*, *Rph12*, *Rph19*/ *Rph1*, *Rph2*, *Rph3*, *Rph5*, *Rph7*, *Rph10*, *Rph11*, *Rph13*, *Rph14*
4652 P^+ [b]	*Rph2*, *Rph4*, *Rph6*, *Rph8*, *Rph9*, *Rph12*, *Rph13*, *Rph19*/ *Rph1*, *Rph3*, *Rph5*, *Rph7*, *Rph10*, *Rph11*, *Rph14*, *Rph19*
4653 P^+ [b]	*Rph1*, *Rph2*, *Rph4*, *Rph6*, *Rph8*, *Rph9*, *Rph12*, *Rph13*, *Rph19*/ *Rph3*, *Rph5*, *Rph7*, *Rph10*, *Rph11*, *Rph14*
5610 P^+ [b]	*Rph4*, *Rph8*, *Rph9*, *Rph10*, *Rph12*, *Rph19*/ *Rph1*, *Rph2*, *Rph3*, *Rph5*, *Rph6*, *Rph7*, *Rph11*, *Rph13*, *Rph14*
5452 P^+ [b]	*Rph2*, *Rph4*, *Rph6*, *Rph9*, *Rph10*, *Rph12*, *Rph19*/ *Rph1*, *Rph3*, *Rph5*, *Rph7*, *Rph8*, *Rph11*, *Rph13*, *Rph14*
5453 P^- [b]	*Rph1*, *Rph2*, *Rph4*, *Rph6*, *Rph9*, *Rph10*, *Rph12* / *Rph3*, *Rph5*, *Rph7*, *Rph8*, *Rph11*, *Rph13*, *Rph14*, *Rph19*
5653 P^+ [b]	*Rph1*, *Rph2*, *Rph4*, *Rph6*, *Rph8*, *Rph9*, *Rph10 Rph12*, *Rph19*/ *Rph3*, *Rph5*, *Rph7*, *Rph11*, *Rph13*, *Rph14*
5673 P^+ [b]	*Rph1*, *Rph2*, *Rph4*, *Rph5*, *Rph6*, *Rph8*, *Rph9*, *Rph10 Rph12*, *Rph19*/ *Rph3*, *Rph5*, *Rph7*, *Rph11*, *Rph13*, *Rph14*

† P^+ and P^- indicate virulence and avirulence, respectively, for differential Prior (*Rph19*), ‡ *Rph15, Rph16*, *Rph17* and *Rph18* not tested, [a] Pathotypes were designated using the nomenclature system outlined by Gilmour (1973), [b] Pathotypes tested against *Rph13*, *Rph14*

Table 2.2. Binary triplets and their corresponding octal numbers used in designating pathotypes of *P. hordei*.

Binary triplet [a]	Octal number
0 0 0	0
0 0 1	1
0 1 0	2
0 1 1	3
1 0 0	4
1 0 1	5
1 1 0	6
1 1 1	7

[a] Three digits represent reaction of differential genotypes grouped into set of three where 0 is susceptible and 1 is resistant.

2.4 Stripe rust of barley

Eriksson and Henning (1894) described the stripe rust pathogen as *P. glumarum*. This name remained valid until Hylander *et al.* (1953) and Cummins and Stevenson (1956) revived the name *P. striiformis* West. Unlike *P. tritici* and *P. graminis*, *P. striiformis* is microcyclic having only the uredinial and telial states. Despite intensive searches, an alternate host for the pathogen has been not reported. Although the stripe rust pathogen has been reported to infect about 320 grass species of about 50 genera, barley can be infected by either the form that infects wheat (*P. striiformis* f. sp. *tritici, Pst*) or the form that infects barley (*P. striiformis* f. sp. *hordei, Psh*).

Psh was known to be prevalent for many years in parts of Western Europe, the Middle East, South Asia, and East Africa (Stubbs, 1985). A highly virulent pathotype of *Psh*, Race-24, was first detected in the South American country of Columbia (Dubin and Stubbs, 1986). From 1976 to 1982, the pathogen was detected in Ecuador, Peru, Bolivia, Chile and Argentina. In 1987, the pathogen was detected for the first time in Mexico, where it caused severe damage to barley, with yield losses of up to 50%. In 1991, barley stripe rust was also reported in the USA, and it has now become a serious disease of barley in parts of California and the Pacific Northwest (Brown *et al.*, 2001). *Psh* has not been reported in Australia. Tests of Australian barley cultivars against Race 24 of *Psh* at CIMMYT Mexico indicated that most lack effective resistance (Wellings, unpublished).

Wellings *et al.* (2000a) reported a new variant of *P. striiformis* causing stripe rust on barley and wild *Hordeum* species in Australia, and regarded it to be different from *Pst* and *Psh* and temporarily designated it Barley Grass Stripe Rust (*BGYR*). This pathogen was highly avirulent on seedlings of most wheat differentials, was partially virulent on the wheat differential 'Chinese 166', and was virulent on a few barley cultivars. Keiper *et al.* (2003) reported further evidence that *BGYR* represented a new *forma specialis*. They found that three different DNA marker types efficiently discriminated *BGYR* from *Pst*. Though most commercial malting grade barley cultivars are resistant to *BGYR*, knowledge of the number of genes for resistance, their mode of inheritance and relationship with resistance to *Psh* is not known and would be useful for future barley breeding efforts.

2.5 Breeding for rust resistance

It was realised long ago that plants differed in their ability to resist disease. Theophrastus noted this in the 3rd Century B.C. and selection of desired plants was a major advance in agriculture. Although knowledge about disease control was scanty, some practices such as eradication of alternate hosts, selection of resistant plants and in ancient times the removal of morning dew droplets with a net or rope proved to be effective in certain circumstances. Mendel (1865) demonstrated the genetic inheritance of characters. The subsequent pioneering studies of Biffen (1905) demonstrated that resistance to wheat stripe rust was also heritable. In the course of this study, Biffen showed that resistance to stripe rust in wheat was governed by a recessive gene that segregated in a typical monohybrid ratio of 3 susceptible: 1 resistant in the F2 generation. Subsequently, several other studies showed that resistance to various diseases was determined genetically. These studies prompted the development of varieties with resistance against major diseases. Resistant varieties have become an economical and eco-friendly approach to tackle major diseases world-wide. Another major landmark in disease resistance breeding was the discovery of Flor's (1956) gene-for-gene relationship. Genetic analysis of resistance in numerous host species and specific virulence in the corresponding pathogen has led to the general acceptance of the gene-for-gene model, which has contributed to the understanding of how host resistance genes interact with corresponding pathogens (Crute and Pink, 1996). This knowledge has been used by most research

groups in applied studies such as pathotype surveys and identifying novel and potentially useful sources of resistance for breeding purposes (Bowder, 1971).

2.5.1 Gene-for-gene theory of host pathogen interaction

Harold Flor (1956) analysed the genetics of the host : pathogen interaction in flax rust and showed that for each host (*Linum usitatissimum*) gene conferring resistance there is a complementary gene conditioning avirulence in the pathogen (*Melampsora lini*). This finding has become widely known as the gene-for-gene hypothesis of host-pathogen interactions. The theory is based on the results of parallel experiments that examined the inheritance of pathogenicity in *M. lini* with respect to the inheritance of disease reaction in flax. Two pathotypes of *M. lini*, contrasting in pathogenicity on a flax cultivar, were intercrossed and F_2 progeny obtained from the cross were inoculated on the cultivar. The segregation pattern for avirulence was typical of a single gene (3 avirulent : 1 virulent). In flax, the inheritance of resistance to *M. lini* was monogenic when F_2 progeny of a cross between two flax cultivars contrasting in disease response were inoculated with the avirulent pathotype of *M. lini*. On the basis of Flor's gene-for-gene hypothesis, the possible interactions between a pair of alleles governing resistance in a plant and the corresponding pair determining pathogenicity in the pathogen can be shown by a quadratic check (Fig. 2.2).

Pathogen genotype	**Host genotype**		
	RR (resistant)	**Rr (resistant)**	**rr (susceptible)**
AA (avirulent)	**Incompatible (LIT)**	**Incompatible (LIT)**	**Compatible (HIT)**
Aa (avirulent)	**Incompatible (LIT)**	**Incompatible (LIT)**	**Compatible (HIT)**
aa (virulent)	**Compatible (HIT)**	**Compatible (HIT)**	**Compatible (HIT)**

Figure 2.2. Interaction between a host resistance gene and a pathogenicity gene and the resulting disease phenotypes. Incompatibility or low infection type (LIT) is the consequence of interaction of the products of the resistance and avirulence alleles whereas compatibility or high infection types (HIT) indicates absence of the interaction.

2.5.2 Molecular basis of the gene-for-gene hypothesis

In gene-for-gene interactions, the induction of the plant defence response that leads to the hypersensitive response (HR) is initiated by the plant's recognition of specific signal molecules (elicitors) produced by the pathogen. These elicitors are encoded directly or indirectly by avirulence genes and R genes are thought to encode receptors for these elicitors. These elicitors are encoded directly or indirectly by avirulence genes and R genes are thought to encode receptors for these elicitors (Fig. 2.3). Elicitor recognition activates a cascade of host genes that lead to HR and inhibition of pathogen growth (Staskawicz *et al.*, 1995).

The HR generally occurs as rapid, localised cell death, and is considered a form of programmed cell death in plants (Heath, 1998). The growth of invading hyphae of the pathogen is obstructed due to dead cells and no further biotrophic interaction is possible. Gene-for-gene systems involving HR have been described for pathosystems including intracellular obligate pathogens (viruses and mycoplasmas) as well as for intercellular facultative and obligate pathogens (bacteria, fungi and nematodes) (Staskawicz *et al.*, 1995). These findings suggest that common or similar recognition and signal transduction mechanisms are operating in different gene-for-gene signaling pathways. In *Phytopthora infestans*, causal pathogen to potato blight disease, number of avirulence (*Avr*) genes were mapped (Van der Lee *et al.*, 1997). Three avirulence genes *Avr*3, *Avr*10 and *Avr*11 were located on the telomeric region of linkage group VIII, and deleting that part of the chromosome resulted in virulence on potatoes carrying the R3, R10 and R11 resistance genes (Van der Lee *et al.*, 2001). The simplest version of the classical receptor-elictor model predicts a direct interaction between the R protein and the corresponding Avr protein. The lack of demonstrable R-Avr interactions in some experiments led to the formulation of the guard hypotheses by Van der Biezen and Jones (1998). This model predicts that R proteins activate resistance when they interact with another plant protein (a guardee) that is targeted and modified by the pathogen to create a favorable environment for the successful infection. Resistance is triggered when the R protein detects an attempt to attack its guardee, which might not necessarily involve direct interaction between the R and Avr proteins. Compelling evidence for this model was recently reported for an Arabidopsis R protein (Mackey *et al*., 2002).

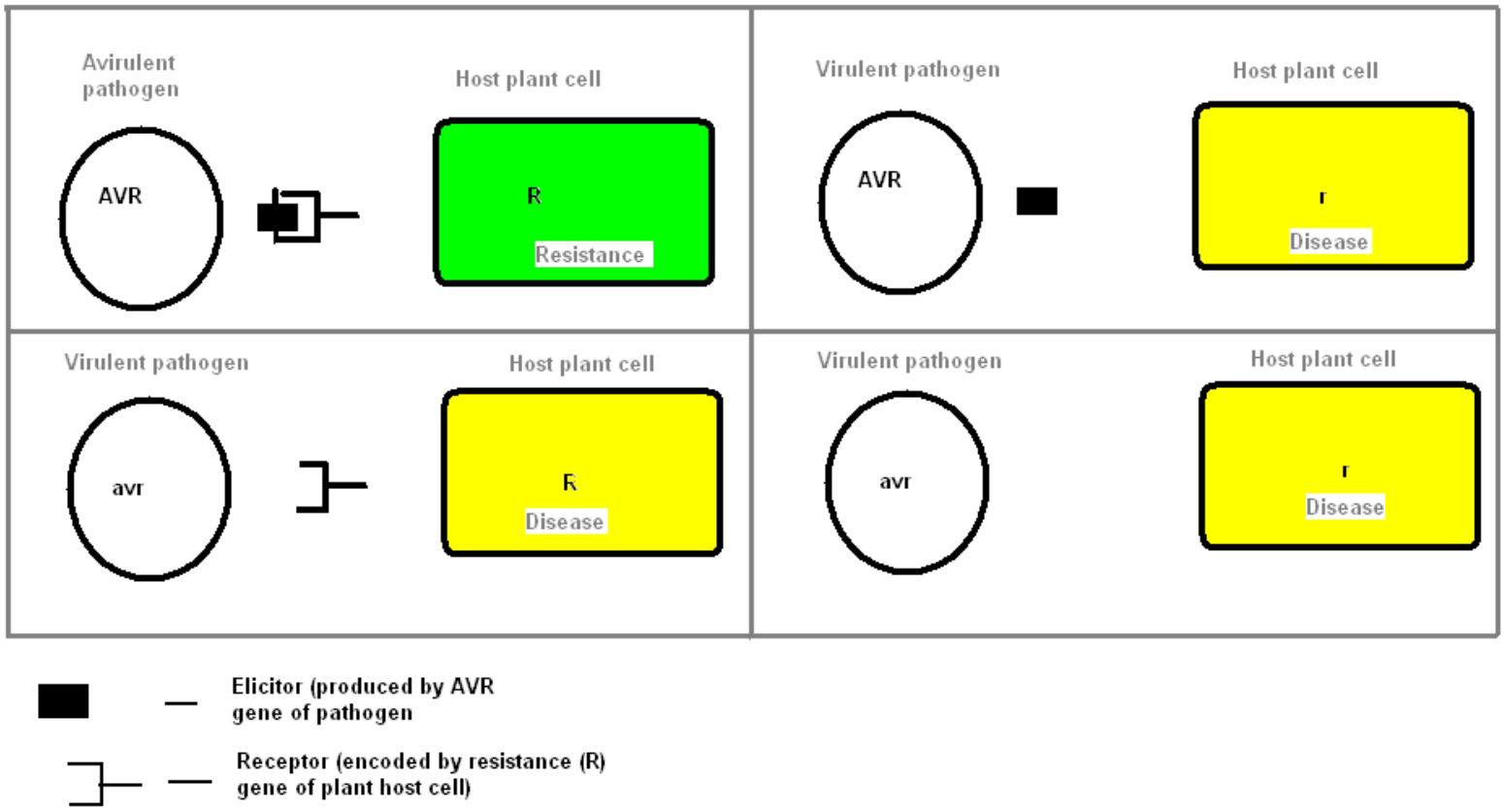


Figure 2.3. Interaction between an elicitor produced by a pathogen and a receptor encoded by a resistance gene of a host plant cell and its effect on disease resistance reaction of the host. Resistance is only expressed when a plant that contains a specific R gene recognises a pathogen that has the corresponding avirulence gene (upper left column). All other combinations lead to lack of recognition by the host, resulting in disease.

2.5.3 Breeding methods

Resistance to disease is often an objective of improving crop plants. However, for many crops it is of lower priority than characters such as yield or quality. Hence, the fundamental objective of rust resistance breeding is to find a method to identify and select the resistance and to combine it with other economically desirable characters to produce a commercially acceptable crop genotype (Lucas, 1998). The first step in achieving host resistance in breeding is to identify a source of resistance. An understanding of the mode of genetic inheritance of the resistance is essential to decide what breeding method is best to incorporate the character into elite germplasm. An analysis of the genetic inheritance of rust resistance involves crossing two parents with contrasting disease response. The hybrid produced and subsequent progeny are tested for rust response. The ratio of resistant to susceptible progeny varies according to the population used, the number of resistance genes segregating, the nature of inheritance of gene/s and the interaction between genes governing the character (Allard, 1960). There are several breeding methods to produce or improve disease resistance in a crop species. In this section, brief descriptions of some of the most common methods used in self pollinated crops are provided.

2.5.3.1 Selection

Selection of a resistant genotype from introduced or locally adapted germplasm is the quickest and cheapest method of producing disease resistant cultivars. Other agronomically important characters can be selected simultaneously. In Australia, Cotterill and Rees (1993) studied the performance of several slow rusting European cultivars. These cultivars gave adequate protection against barley leaf rust in Queensland and one cultivar, ‘Koru’, was reselected and released locally as the cultivar ‘Gilbert’.

2.5.3.2 Mutation

The role of induced mutation in disease resistance breeding is limited although it has sometimes provided valuable sources of resistance (Mick, 1990). These include *Ascochyta* blight resistance in chickpea, *Sclerospora graminicola* resistance in pearl millet and *Verticillium* wilt resistance in peppermint. The durable

powdery mildew resistance gene *mlo* was obtained independently from several barley mutant lines. This gene was subsequently found in untreated barley populations, suggesting that so called 'induced mutations' for disease resistance may occur naturally at a very low frequency (Jørgensen, 1992).

2.5.3.3 Hybridisation

Various hybridisation techniques are available for breeding disease resistant cultivars. The technique used will depend on the reproductive system of the crop plant, the number of genes governing resistance and the mode of inheritance of the character.

2.5.3.3.1 Pedigree method

The pedigree method was first described by Love (1927). It was developed from the pure-line system and has become widely used for self-pollinated crops. In the pedigree method, plants with superior agronomic traits are selected as early as the F_2 generation, where maximum heterozygosity is present. Following initial selection, plants are reselected in each subsequent generation until a reasonable level of genetic homozygosity is reached and plants appear phenotypically homogeneous. The pedigree of each selection is maintained by a numbering system so that parent-progeny relationships can be traced back to an individual F_2 plant in subsequent generations.

2.5.3.3.2 Backcrossing method

The backcross breeding method was suggested by Harlan and Pope (1922) and has been used widely to improve superior cultivars that otherwise lack traits such as disease resistance. The method is suited to transferring highly heritable traits that are governed by 1 to 3 genes from a donor parent to a recurrent parent. The recurrent parent is crossed with the donor parent and the resultant F_1 is again backcrossed to the recurrent parent. The trait of interest from the donor parent is maintained by selection. Plants selected in the next generation are repeatedly backcrossed with the recurrent parent until all desired features of the recurrent parent plus the trait of interest from the donor parent are recovered.

2.5.3.3.3 Bulk selection/ F2 progeny method

Bulk breeding is an economical alternative to the pedigree method. It is based on natural selection. Following hybridization and selfing of the resulting F_1s, several hundred F_2 seeds are planted and plants are harvested in bulk. The procedure is repeated until the F_4 generation when seeds are space planted to facilitate artificial selection for single superior plants. Selected plants are grown as family seed increase plots for a F_7 yield trial. The method is also useful to incorporate quantitative disease resistance.

2.5.3.3.4 Doubled haploid method

In barley, haploid plants can be achieved from the F_1 generation by culturing anthers and/or microspores *in vitro* or via a conventional approach by interspecific crosses with *H. bulbosum*. Both methods have been used in commercial barley breeding programmes. Homozygous fertile plants are obtained from haploids by doubling the chromosome number. Though spontaneous doubling of chromosome is common in barley haploids, colchicine treatment is required to restore the fertility of many haploid plants (Devaux, 1988). This strategy produces a population that is homozygous at all loci, after just one generation. Doubled haploid lines have undergone only one meiotic cycle and carry a completely homozygous chromosome set. This means that the genetic information per plant is constant and unlimited testing of progeny can be conducted over many environmental locations. Doubled haploids can be used to study gene action and interaction, estimate the number of genes, calculate combining abilities, to detect of gene linkages or pleiotropy, and in mapping studies.

2.6 Disease assessment

A disease assessment scale is essential to compare resistance across genotypes and in permitting phenotypes to be separated into resistance groups in genetic studies. Different scales have been developed by rust workers to assess rust response (Table 2.3 and Table 2.4). Assessment can be qualitative, quantitative or a combination of both. Disease can be rated either at the seedling stage in controlled environments or at the adult stage under field conditions. Stakman and co-workers developed a scale to assess rust diseases on seedlings on the basis of infection type. The original scale developed by Stakman *et al.* (1962) for rating leaf rust and stem

rust at seedling growth stages has been used widely. The scale is also useful for scoring adult plants. However, Cobb (1892) published a diagrammatic rust scale to assess rust severity at adult plant growth stages in the field, in which 100% rust severity was assigned when 50% of the leaf area was covered with rust pustules. Peterson *et al.* (1948) proposed another scale that scored 100% rust severity when rust pustules covered 37% of the leaf surface area. This was based on the observation that at this stage, development and destructiveness of the underlying mycelium was maximum. The Cobb scale or the modified Cobb scale rating by the disease response (R, no uredinia present; Tr, trace or minute uredinia on leaf without sporulation; MR, small uredinia with slight sporulation; MR-MS, small to medium size uredinia with moderate sporulation; medium size uredinia with moderate to heavy sporulation, S, large uredinia with abundant sporulation, uredinia often coalesced to form lesions) has been used commonly by many laboratories to assess disease under field conditions (Roelfs *et al.*, 1992). The scale originally developed by Stakman and co-workers for wheat stem rust has been adapted to most of the cereal rusts except stripe rust, which infects systemically. The most commonly used scale for stripe rust is that developed by Gassner and Straib (1932). This scale was unsuitable for scoring adult plants, and accordingly, the McNeal *et al.* scale (1971) was developed. Disease at the adult plant stage can also be measured by using area under the disease progress curve (AUDPC). Although this method is very labour intensive, it has the advantage of being able to score disease across genotypes that differ in maturity.

Table 2.3. Major infection types used to assess stem rust and leaf rust response at seedling growth stages.

Infection type	Host response	Symptoms
0	Immune	No visible uredinia
;	Very resistant	Hypersensitive flecks
1	Resistant	Small uredinia with necrosis
2	Resistant to moderately resistant	Small to medium sized uredinia with green islands and surrounded by necrosis or chlorosis
3	Moderately resistant/moderately susceptible	Medium sized uredinia with or without chlorosis
4	Susceptible	Large uredinia without chlorosis
X	Resistant	Heterogeneous, similarly distributed over the leaves
Y	?	Variable size with larger uredinia towards the tip
Z	?	Variable size with larger uredinia towards the leaf base

Table 2.4. Major infection types used to assess stripe rust response.

Infection type	Host response	Symptoms
0	Immune	No visible uredinia
1	Very resistant	Necrotic flecks
2	Resistant	Necrotic areas without sporulation
3-4	Resistant	Necrotic and chlorotic areas with restricted sporulation
5-6	Moderately resistant	Moderate sporulation with necrosis and chlorosis
7-8	Moderately susceptible	Sporulation with necrosis
9	Susceptible	Abundant sporulation without chlorosis

2.6.1 Leaf rust assessment with respect to yield loss in barley

The flag leaf stage is considered an important contributor to final yield. Any interference in photosynthesis, water balance and food transportation during grain fill can affect grain size and weight. King and Polly (1976), Melville *et al.* (1976) and Udeogalanya and Clifford (1982) observed yield losses in the range of 0.60 to 0.77% due to 1% increments of rust on the flag leaf, while lower yield losses (0.4%) were observed due to 1% increments of rust on whole plants (King and Polly, 1976). Teng *et al.* (1979) attempted to correlate yield losses with different assessment methods based on 1) a single assessment of disease at the flag leaf stage (Critical Point, CP) 2) several assessments of disease (Multiple Point, MP) and 3) measurement of disease profile from AUDPC. All models were satisfactory in explaining the yield loss. However of the assessment methods used, the multiple point (MP) was the best and explained 90% of the total variation, followed by AUDPC models (58.2-62.2%) and various CP models (45.3-62.2%).

2.7 Resistance

Resistance is defined as the ability of a host plant to hinder the growth and or development of a pathogen. Researchers working on host : pathogen interactions have had difficulty in agreeing on a common set of terms to describe resistance. In the present review, attempts are made to describe some of the most commonly used classifications of host resistance of cereal crops to rust diseases. In broad terms, resistance to rust pathogens can be classified based on growth stage (adult plant resistance versus seedling resistance), genetics (major genes versus minor genes) and durability (durable versus non durable).

2.7.1 Classification of resistance on the basis of growth stage

Resistance to rust diseases can be categorised on the basis of the growth stage at which it is expressed. Seedling resistance is expressed at early growth stages and often remains effective throughout all growth stages. On the contrary, resistance that expresses in the adult stage or post seedling stage only is called adult plant resistance (APR). However, this demarcation is not absolute and there are some sources of APR that are expressed at seedling growth stages under certain environmental conditions, or genetic background and/or against certain pathotypes. Gene *Lr34* has been classified as APR (Dyck, 1987) but it can be identified at the seedling stage with certain pathotypes

under low temperature and light (Drijepondt *et al.*, 1991). A similar situation was observed with *Lr13*, which was originally classified as APR (Dyck *et al.*, 1966). In these cases, knowledge of the exact growth stage, environmental conditions and pathotype for the expression of APR can avoid laborious field procedures to evaluate resistance, and reduce the time needed for the breeding cycle.

A number of APR genes (*Lr12, Lr13, Lr22a, Lr22b, Lr34* and *Lr35*) against wheat leaf rust, *Sr2* against wheat stem rust and *Yr16* and *Yr18* against wheat stripe rust have been characterised in wheat (McIntosh *et al.*, 1995). Experience with APR in wheat against rust pathogens has shown that APR is often an important component of durable resistance. However, Park and McIntosh (1994) reported pathotypes of *P. triticina* with virulence for the APR genes *Lr12, Lr13* and *Lr22b* in Australia.

2.7.2 Classification of resistance on a genetic basis

Major gene(s) resistance is usually governed by one or two genes and is also known as qualitative resistance due to the clear and distinct phenotypes produced. Major gene resistance is known to be associated with the hypersensitive reaction and often shows race-specificity. Resistance that can not be classified into clear discrete classes and shows continuous variation is called minor gene(s) resistance and is usually governed by several genes which alone produce small phenotypic effects. Continuous variation can be caused by the environment and/or segregation of several loci, each having a minor effect on the resistance. This resistance is also called quantitative resistance. The inheritance of both qualitative and quantitative resistance follows the laws of Mendel.

2.7.2.1 Major gene/qualitative resistance

The major discoveries of the genetic inheritance of characters by Mendel (1865), the genetic basis of resistance by Biffen (1905), physiological specialisation in a rust pathogen by Stakman and Levine (1962), and the concept of gene-for-gene interaction by Flor (1956), have all helped breeders to develop plant genotypes with major gene resistance. The ease with which major genes can be incorporated and the high level of protection conferred by them are the major reasons for the wide use of this approach. Major gene resistance dominated disease resistance breeding for more than seven decades, and continues to be a significant approach to rust resistance breeding. However, often cultivars with single resistance genes give temporary

protection, lasting only until the occurrence of a new mutant pathotype, the increase of virulent pathotypes already present in the pathogen population, or the introduction of a virulent pathotype. In this context, combining genes could provide longer lasting resistance as it would require pathogen genotypes to undergo multiple simultaneous changes in order to become virulent.

2.7.2.1.1 Multilines

Land races are a mixture of several lines that are not genetically homogeneous and are usually not prone to rust epidemics. The endurance of land races to epidemic conditions was assumed to be due to the large genetic variation among the population, which prevents perpetuation of rust pathotypes. The concept of land races has been utilised in producing multilines. Jensen (1952) and Borlaug (1953) proposed the use of multilines to control stem rust in wheat. Multilines are mixtures of near isogenic lines that differ only in the resistance gene present. Multilines have been developed and released in wheat and oats. However, the mechanisms operating in multilines that protect against disease are different in different in pathosystems. Any reduction in disease development may be due to a reduction in the spatial density of susceptible plants, whereby susceptible plants are protected by surrounding plants carrying resistance genes. Disadvantages with multilines include the amount of effort needed and the lengthy breeding procedures needed to develop them. Moreover, multilines may not always be popular among growers. Nevertheless, the multiline cultivars “Miramar 63” and “Miramar 65”, resistant to wheat stem rust, and the oat multiline cultivar “Dirty” for control of crown rust, were released for cultivation in Iowa. A wheat multiline having 16 leaf rust resistance genes has also been produced by CIMMYT.

2.7.2.1.2 Pyramiding or combining resistance genes

Unlike multilines, pyramiding resistance genes is based on the theory of combining several undefeated resistance genes in a single genotype in such a way that every individual of the cultivar population possesses these genes. This concept was effective in controlling leaf rust in wheat with combinations involving *Lr13* or *Lr34* (Kolmer *et al.*, 1991). Wheat cultivars possessing about five to six stem rust resistance genes have been released i.e. ‘Mendos’, ‘Egret’, ‘Gamut’, ‘Timgalen’ and ‘Gatcher’ (Luig, 1983). Examples of the development of virulence matching

complex gene structure are also available. The wheat cultivar 'Cook', with stem rust resistance genes *Sr5*, *Sr6*, *Sr8a* and *Sr36*, was rendered susceptible by the development of a pathotype with matching virulence (Zwer *et al*., 1992).

2.7.2.2 Minor genes/quantitative resistance

In contrast to major gene resistance, minor gene or quantitative resistance often shows continuous variation. The confounding effect of several genes and/or the environment makes it difficult to separate individuals into discrete classes in this type of resistance. The inheritance of quantitative characters is not easily studied using classical techniques and the development of biometrical techniques has greatly shifted efforts to understand quantitative resistance. There are conflicting opinions about quantitative and qualitative resistance in terms of durability. Given that many genes are involved in quantitative resistance, the chances that a pathogen may mutate and acquire virulence matching all resistance genes are low and hence this resistance is assumed to be durable. However, according to Johnson (1984), quantitative inheritance may not necessarily be a factor in the durability of resistance.

2.7.2.2.1 Partial resistance

There is a vast body of literature dealing with partial resistance to *P. hordei* in barley. Partial resistance was first reported in potato against *Pythopthora infestans* (Schaper, 1951; Deshmukh and Howard, 1956; Van Der Zaag, 1959; Van der Plank, 1963, 1968). These reports were extended by Parlevliet and Ommeren (1975), who showed similar resistance in barley. According to Parlevliet and Ommeren (1975), partial resistance is distinct from seedling resistance and adult plant resistance, which both operate on hypersensitive reaction and are usually race-specific. However race-specificity of partial resistance to different isolates was reported by Qi *et al.* (1999). Recent cytological studies on the interaction between *P. infestans* and *Solanum* suggested that the hypersensitive response (HR) was common in both major resistance and partial resistance as part of the defence response. Potato clones carrying major resistance genes showed the HR within 22 h of infection, whereas in partially resistant clones the HR was induced between 16 to 46 h (Vleeshouwers *et al.*, 2000).

In partial resistance, the host is susceptible at all growth stages but the infection frequency, latent period, rate of spore production and period of spore production may vary. Selection for partial resistance is often difficult in field plots as all genotypes

show a susceptible reaction (Parlevliet and Ommeren, 1975). The epidemic development of disease within the growth cycle of the host is determined by the initial amount of disease and the rate at which the disease increases (Van der Plank, 1963). According to this hypothesis, an epidemic can be avoided by reducing the reproductive rate of the pathogen. Van der Plank (1963) showed that infection frequency, latent period, sporulation rate and infectious period are components of partial resistance.

Neervoort and Parlevliet (1978) studied the components of partial resistance to leaf rust in eight barley cultivars. They observed substantial variation among the cultivars for each component. Among these components, latent period was found to be the most crucial factor in partial resistance. In a further study, Parlevliet (1978) reported that latent period was governed by many genes that were additive in nature. Based on latent period, several west European cultivars were shown to have variable levels of partial resistance to *P. hordei* (Parlevliet *et al.*, 1980). Histological studies on partial resistance in the barley cultivar 'Vada' demonstrated early abortion of hyphal growth of fungal spores at adult plant growth stages, in contrast to seedling growth stages (Parlevliet and Kievit, 1986).

2.7.3 Classification of resistance on the basis of durability

Irrespective of race specificity, Johnson (1978, 1981) coined the term "durable resistance" to refer to resistance that remained effective in a cultivar grown for many years in wide array of environments in the presence of the pathogen. Durable resistance in cultivars can be either simple major gene resistance or complex polygenic resistance. The classic examples of durable resistance due to single genes are S*r2* in wheat against wheat stem rust and *Lr34/Yr18* in wheat against wheat leaf rust/stripe rust. Another example of durable resistance conferred by a major gene is the *mlo* gene in barley against powdery mildew. This resistance gene was introduced into several cultivars and has provided complete resistance against powdery mildew in northern Europe (Jørgensen, 1992). Similarly, increased durability of resistance has been reported by assembling and deploying multiple major resistance genes in wheat against stem rust in Australia (McIntosh *et al.*, 1995). Durability of resistance can be achieved by a thorough knowledge of the genetics of host resistance, population genetics, and evolutionary biology of the pathogen and interaction of crop management practices with host resistance. McIntosh and Brown (1997) found that a gradual increase in the area occupied by wheat cultivars equipped with durable resistance to stem rust in the

summer rainfall areas of northern New South Wales and Queensland resulted in an appreciable reduction in pathogen population size and variability.

2.8 Major gene resistance to leaf rust in barley

The first study of the genetics of resistance to leaf rust in barley was published in 1927, when Waterhouse studied the inheritance of resistance to leaf rust in six Australian barley cultivars viz. 'Californian feed', 'O.A.C. 2', 'Cape', 'Manchuria', 'Minn. II 21.15' and 'Minn. 21.17'. He showed that the resistance in all was due to monogenic dominant genes. In a second study, Waterhouse (1947) demonstrated that the resistance genes present in the six barley cultivars were at the same locus. Watson and Butler (1947) showed that the genes for resistance to leaf rust in 'Minn. II 21.15' and 'No. 22' were different and not allelic. They designated these two genes *Pa1* and *Pa2*, respectively. 'Oderbrucker', a differential genotype used by Waterhouse (1947), had a gene at the same locus as 'Minn. II 21.15' (Watson and Butler, 1947). Henderson (1945) designated two genes *Pa* and *Pa1*. He showed that the varieties 'Weider', 'Bolivia', 'Purple Nepal', 'Modia', 'Morocco', 'Barley 305', 'Ricardo' and 'Marco' had a common single gene (*Pa;* now *Rph2*) for resistance to leaf rust, while the variety 'Estate' had gene *Pa1* (*Rph3*). Recent studies, combined with previous studies, have suggested that *Rph2* is a complex locus comprising many alleles (Franckowiak *et al.*, 1997). Roane (1962) conducted a series of genetic studies to determine the number of loci conditioning leaf rust reaction in nine North American differential varieties. He identified four loci and designated them tentatively A, B, C and D. 'Reka I' and 'Bolivia' possessed the A locus in common, while 'Quinn' possessed both the A and the B loci. 'Oderbrucker', 'Speciale' and 'Sudan' possessed locus C, and locus D was present in the differential genotypes 'Gold' and 'Lechtaler'. Resistance to leaf rust in barley has also been described by several other workers (Zloten, 1952; Starling 1956; Moseman and Greeley, 1965). However, the relationships between the genes identified in these studies were not resolved.

A series of experiments was conducted by Roane and Starling (1967, 1969, and 1970) to resolve the genetic relationships between seedling resistance genes that had been identified by previous workers. On the basis of genetic relationships, they described a series of genes, *Pa1* to *Pa6*, in the barley differential set based on the results of reaction to race 4 (isolate 57-19) of *P. hordei*. The genes were given the

designation "*Pa*" because at that time, *P. hordei* was referred to as *P. anomala*. Following the adoption of the name *P. hordei*, Moseman (1972) suggested changing the gene symbols *Pa1* - *Pa6* to *Rph1* - *Rph6*. 'Bolivia' was shown to carry two loci i.e. *Rph2* and *Rph6* for resistance to *P. hordei* (Roane and Starling, 1967). Zhong *et al.* (2003) separated the *Rph6* locus of 'Bolivia' with the help of pathotype ND8702. The locus was positioned on chromosome 3HS and shown to be allelic to the *Rph5* locus of 'Magnif 104' (Zhong *et al.*, 2003).

Resistance gene *Rph7* was identified in the North African cultivar 'Cebada Capa' (Starling, 1956; Johnson, 1968; Dillard and Brown, 1969). This gene was considered to be at the same locus as *Rph5* in 'Quinn' (Roane and Starling, 1970). Johnson (1968), however, indicated that 'Cebada Capa' carried a dominant gene that differed to all genes from *Rph1* to *Rph6*. This gene was designated as *Pa-y*, and was thought to be similar to the dominant gene present in 'Forrajera Klein', 'La Estanzuela' and 'H2212'. Frecha (1970, 1971) studied linkage relationships between *Pa5* and *Pa-y*. He reported that the *Pa5* resistance locus of 'Quinn' was closely linked to the *Pa-y* resistance locus of 'Forrajera Klein', with a recombination value of approximately 8%. However, genetic analysis of resistance in 'Cebada Capa', 'La Estanzuela', 'H2212' and 'Forrajera Klein' suggested that they all carried *Rph7* (Parlevliet, 1976a). Yahyaoui *et al.* (1988) reported new sources of resistance to *P. hordei* in the Tunisian landraces 'Tu17', 'Tu27' and 'Tu34'. Genetic analysis and allelism tests between 'Tu17' and a stock carrying *Rph7* suggested that the gene carried by 'Tu17' is an allele of *Rph7* (Chicaiza *et al.*, 1996). The temperature sensitivity of *Rph7* was studied by Clifford and Udeogalanya (1976). A complete compatibility of pathogen on host carrying *Rph7* was observed at a very low temperature (5^{o}C), while host plants were resistant to the same pathogen isolates at high temperatures. This characteristic could be useful in selecting genotypes possessing *Rph7* in combination with other *Rph* genes, where *Rph7*-virulent pathotypes are not available.

The allele symbols *Rph8* (Tan, 1977a) and *Rph9* (Clifford and Udeogalanya, 1976, Tan, 1977b) were designated to the loci conferring resistance against *P. hordei* in 'Egypt 4' and 'Hor2595' ('CI 1243'), respectively. It was speculated that *Rph9* might be similar to the resistance found in the German cultivar 'Trumpf' (also known as 'Triumph') (Walther and Lehmann, 1980). Further tests with different isolates suggested that *Rph9* and 'Triumph' exhibited different infection types. A genetic analysis of 'Triumph' implied that the resistance was governed by three genes (two

dominant and one recessive) (Walther, 1987). In another study, a single resistance gene was identified in 'Triumph' and designated *Rph12* (Jin *et al.*, 1993). The relationship between *Rph9* and *Rph12* was subsequently resolved by Borovkova *et al.* (1998), who proved that *Rph12* and *Rph9* are allelic (Table 2.5).

Feuerstein *et al.* (1990) described two leaf rust resistance loci derived from *H. spontaneum* that had been backcrossed into cv. 'Clipper'. These loci were different from other reported *Rph* genes and were designated *Rph10* and *Rph11*. Jin *et al.* (1996) studied inheritance of leaf rust resistance in four barley accessions ('PI 531840', 'PI 531841', 'PI 531849' and 'PI 584760') and their allelic and linkage relationships with other *Rph* genes. The resistance in each accession was governed by a single locus. An incomplete dominant inheritance was observed in accessions 'PI 531841' and 'PI 584760', while a completely dominant inheritance was observed in 'PI 531840' and 'PI 531849'. Allelism tests between 'PI 531841' and 'PI 531840' suggested that the same resistance locus was present in both and that it was allelic to *Rph2*. The linkage relationships with other *Rph* genes indicated that the locus providing resistance in 'PI 531841' and 'PI 531840' was linked with *Rph5* with recombination frequencies of 33.8 ± 3.8 and 17.0 ± 3.5%, respectively. This contrasts with the results of molecular mapping of *Rph5* and *Rph2* that showed *Rph5* was located on short arm of barley chromosome 3H (Mammadov *et al.*, 2003) and that *Rph2* was located on the short arm of chromosome 5H (Borovkova *et al.* 1997; Franckowiak *et al.*, 1997). The resistances in 'PI 531849' and 'PI 584760' were not allelic to previously identified loci. New allele symbols, *Rph13* and *Rph14*, were therefore given to the resistances in 'PI 531849' and 'PI 584760', respectively.

Jin *et al.* (1995) identified several potential sources of resistance to *P. hordei* in *H. spontaneum* accessions ('PI 354937', 'PI355447', 'PI 391024', 'PI 391069', 'PI 391089', 'PI 466245', and 'PI 646324'). Genetic studies of these accessions demonstrated a common single locus governing resistance against *P. hordei*. The locus was not allelic to previously identified loci, and was given the new allele symbol of *Rph15* (Chicaiza *et al.*, 1996). Ivandic *et al.* (1998) reported a new gene in two accessions of *H. spontaneum*. The gene was effective against a wide range of *P. hordei* pathotypes, including several from Israel, Morocco and United States that were virulent on *Rph7*. This gene was designated *Rph16* and it was mapped to chromosome 2HS. Recent molecular and allelism studies revealed that *Rph15* and *Rph16* are allelic (Weerasena *et al.*, 2004).

Table 2.5. Recommended locus and allele symbols for genes conferring resistance to *Puccinia hordei* in barley.

Gene symbol†	Source	Country of origin	Species of origin	Reference(s)
Rph1.a	Oderbrucker	Manchuria	*H. vulgare*	Henderson (1945); Waterhouse (1948)
Rph2.b	Peruvian	Peru	*H. vulgare*	Levine and Cherewick (1952); Starling (1956); Steffenson and Jin (1997)
Rph2.j	Batna	Algeria	*H. vulgare*	Reinhold and Sharp (1982); Starling (1956); Steffenson and Jin (1997)
Rph2.k	Weider	Australia	*H. vulgare*	Henderson (1945); Sharp and Reinhold (1982); Watson and Butler (1947); Steffenson and Jin (1997)
Rph2.l	Juliaca	Peru	*H. vulgare*	Levine and Cherewick (1952); Starling (1956)
Rph2.m	Kwan	India	*H. vulgare*	Henderson (1945); Zloten (1952); Steffenson and Jin (1997)
Rph2.n	Chilean D	?	*H. vulgare*	Levine and Cherewick (1952); Tan (1977b)
Rph2.q (*Rph5*.e)	Quinn	Australia	*H. vulgare*	Roane and Starling (1967); Starling (1956)
Rph2.s	Ricardo	Uruguay	*H. vulgare*	Henderson (1945); Moseman and Roane (1959); Zloten (1952)
Rph2.t	Reka 1	Australia	*H. vulgare*	Levine and Cherewick (1952); Starling (1956); Moseman and Greeley (1965)
Rph2.u	Ariana	Tunisia	*H. vulgare*	Sharp and Reinhold (1982); Zloten (1952)
Rph2.y	PI 531841	?	*H. spontaneum*	Jin *et al.* (1995); Jin *et al.* (1996)
Rph3.c	Estate	Egypt	*H. vulgare*	Henderson (1945); Roane and Starling (1967)
Rph3.w	PI 466324	-	*H. spontaneum*	Jin and Steffenson (1994); Chiciaza *et al.* (1996)
Rph3.aa	PI 584765	CIMMYT	*H. vulgare*	Jin (unpublished).

Rph4.d	Gold	Sweden	*H. vulgare*	Moseman and Reid (1961); Roane (1962)
Rph5.e	Magnif 104	Argentina	*H. vulgare*	Roane and Starling (1967); Starling (1956); Frecha (1970); Yahyaoui and Sharp (1987)
Rph6.f (*Rph2*.r)	Bolivia	North Africa	*H. vulgare*	Henderson (1945); Roane and Starling (1967); Starling (1956)
Rph7.g	Cebada Capa	North Africa	*H. vulgare*	Johnson (1968); Nover and Lehmann (1974); Parlevliet (1976); Starling (1956)
Rph7.ac	Tu 17a	Tunisia	*H. vulgare*	Chicaiza *et al.* (1996)
Rph8.h	Egypt 4	Egypt	*H. vulgare*	Levine and Cherewick (1952); Tan (1977a)
Rph9.i	Hor 2596	Ethiopia	*H. vulgare*	Clifford and Udeogalanya (1976); Tan (1977a)
Rph10.o	Clipper BC8	-	*H. spontaneum*	Feuerstein *et al.* (1990)
Rph11.p	Clipper BC67	-	*H. spontaneum*	Feuerstein *et al.* (1990)
Rph12.z (now 9.z)	Triumph	Germany	*H. vulgare*	Walther (1987); Jin *et al.* (1993)
Rph13.x	PI 531849	-	*H. spontaneum*	Jin and Steffenson (1994); Jin *et al.* (1996)
Rph14.ab	PI 584760	Egypt	*H. vulgare*	Jin *et al.* (1996)
Rph15.ad	PI 355447	-	*H. spontaneum*	Chicaiza *et al.* (1996)
Rph16.ae	HS078 HS084	-	*H. spontaneum*	Ivandic *et al.* (1998)
Rph17 [a]		Australia	*H. bulbosum*	Pickering *et al.* (1998)
Rph18 [a]		Australia	*H. bulbosum*	Pickering *et al.* (2000)
Rph19.ah	Prior	Australia	*H. vulgare*	Park and Karakousis (2002)

† The locus and allele symbols suggested by Franckowiak *et al.* (1997), [a] tentative allele symbols.

Pickering *et al.* (1997, 2000) intercrossed a colchicine induced autotetraploid *H. bulbosum* (accession 'HB2032') with diploid *H. vulgare* (cv. 'Emir') and the resulting partially fertile triploid hybrid was backcrossed to 'Emir'. The recombinants obtained by this method were assessed for resistance to leaf rust. Two introgressions of *H. bulbosum* chromatin conferred resistance to leaf rust. The resistance loci in the stocks were designated *Rph17* and *Rph18*, respectively.

An unknown resistance gene present in the differential cultivar 'Reka 1' (Tan, 1977a) and several other Australian cultivars also present in 'Prior' (Cotterill *et al.*, 1994), was characterised by Park and Karakousis (2002). This locus was designated *Rph19*, and was mapped on chromosome 7HL. It was shown to be linked with *Rph3* with a recombination distance of 28 ± 4.3 cM.

Resistance to *P. hordei* in Australian barley cultivars was first reported in 1927 (Waterhouse, 1927), following a leaf rust epidemic in northern NSW. However very little documentation was available on leaf rust of barley until further epidemics were experienced from 1970 to 1990. Cotterill *et al.* (1994) tested seedlings of 38 Australian commercial barley cultivars with 11 pathotypes of *P. hordei* and in the field at adult plant growth stages against two pathotypes. Based on infection types at seedling and adult plant growth stages, each cultivar was postulated to carry one or two known resistances genes (*Rph2*, *Rph4* and *Rph12*) and/or uncharacterised resistance. Pathotypes virulent on all of the genes postulated were identified in Australia (Cotterill *et al.*, 1994). In a subsequent study, Cotterill *et al.* (1995) reported that most charcaterised genes were ineffective against pathotypes identified in Australia during 1966 to 1995, and only *Rph3* and *Rph7* were considered to be suitable for protecting Australian barley cultivars from the disease (Cotterill *et al.*, 1995). In a more recent study, Park (2003) reported that in addition to *Rph3* and *Rph7*, the newly described genes *Rph11*, *Rph14*, *Rph15* and *Rph18* were also effective under Australian conditions with prevailing pathotypes. However, pathotypes virulent to *Rph3* were detected in New Zealand (Cromey and Villjanen-Rollinson, 1995). Although *Rph7* has provided resistance against leaf rust in Europe, virulence for *Rph7* has been identified in Israel (Golan *et al.*, 1978), Morocco (Parlevliet *et al.*, 1981) and North America (Steffenson *et al.*, 1993). Virulences for *Rph11* and *Rph14* have also been found frequently in many parts of the world (Fetch *et al.*, 1998). Previous experience has shown that resistance based on single genes is unlikely to remain effective for a long time. Because pathotypes virulent on *Rph3*,

Rph7, *Rph11* and *Rph14* have been reported, it is not recommended to use them alone in a susceptible genetic background (Park, 2003). In this context, pyramiding two or more genes or incorporating APR genes could increase the durability of resistance. Several European barley cultivars, reputed for their slow rusting characteristics, were resistant under Australian conditions and could be useful sources of resistance in current efforts of controlling leaf rust epidemics in barley (Cotterill *et al.*, 1992, 1994; Park, unpublished data).

2.9 Classical and molecular mapping of disease resistance

In barley, the earliest approaches to locate disease resistance genes were trisomic analysis and linkage with other characterised traits or morphological analysis. Trisomic analysis was used to locate the leaf rust resistance genes *Rph1*, *Rph4* and *Rph5*, whereas linkage analysis was used to locate *Rph13* and *Rph3* (Table 2.6). Though these methods of mapping genes have served well in various types of basic research, their use in applied plant breeding has been very limited. Both methods have been circumvented largely by the advent of isozyme and DNA based molecular markers.

The introduction of molecular markers has made it easier to map, characterise and select for disease resistance genes in crop species. Molecular markers are simply landmarks on chromosomes that serve as reference points in locating other genes of interest once a genetic map is constructed. Molecular markers are classified broadly into four groups: enzyme-based markers (isozymes), hybridisation based DNA markers; Polymerase Chain Reaction (PCR) based markers; and DNA chip and sequence-based markers. The choice of marker system is based on the objective and the cost involved per assay. Practical application of markers started with the development of chemical assays for isozymes that detect variations in protein products. With the discovery of restriction enzymes, the first DNA based marker technique, RFLPs (Restriction Fragment Length Polymorphisms), was developed (Botstein *et al.*, 1980). Since then, several other molecular markers systems viz. RAPD (Random Amplified Polymorphic DNA) (Williams *et al.*, 1990), AFLP (Amplified Fragment Length Polymorphism) (Vos *et al.*, 1995), SSR (Simple Sequence Repeat) (Tautz and Renz, 1989), SNP (Single Nucleotide Polymorphism) (Brookes, 1999), and RGAP (Resistance Gene Analogue Polymorphism) have been

developed. Among these, AFLP, SSR and RGAP techniques have been used frequently in mapping disease resistance genes in barley (Chelkowski *et al.*, 2003).

2.9.1 Marker Assisted Selection

The most promising and widely cited benefit of molecular markers in plant improvement is for Marker Assisted Selection (MAS) (Masojc, 2002). Molecular markers can be potentially utilised to select plants with desirable traits on the basis of genetic assays. Given the lengthy breeding cycle and considerable resources needed to incorporate disease resistance, molecular markers for specific traits can significantly improve the efficiency of resistance breeding. Markers allow the simultaneous incorporation of multiple resistance genes (pyramiding resistance genes) against one or more pathogens into elite germplasm. Molecular markers have been successfully utilised to pyramid major resistance genes to a single pathogen in a single genotype (Singh *et al.*, 2001; Hittalmani *et al.*, 2000) and against multiple pathogens (Datta *et al.*, 2002). Mohler and Singrun (2004) outlined three issues of importance in applying molecular markers successfully: 1. Markers should co-segregate or map as close as possible to the target gene (within 2 cM), in order to have low recombination frequency between the target gene (a better estimate of genetic distance between the target locus and the marker could be obtained by validating the marker on other populations segregating for the same gene, and, the accuracy of MAS will be improved if two markers flanking the target locus are used). 2. The marker should detect polymorphism between genotypes with or without the target locus. 3. Cost effective simple PCR markers are required for rapid genotype screening of a large population.

2.9.2 Tagging or mapping major genes

Mapping major resistance genes is an important step in establishing their identities and their allelic relationships with known disease resistance genes. Genetic populations developed, such as F_2, F_3, BC_1, SSD (Single Seed Decent), RIL (Recombinant Inbreed Lines) and DH, are commonly used in gene mapping studies. A genetic map of the population is constructed using markers that are polymorphic between the parents, and the population can be scored for segregation of traits such as a major resistance genes. The linkage between marker loci and a major resistance gene is estimated by converting recombination frequency into genetic distance. This

method was originally used to develop the world's first genetic map in *Drosophila melanogaster* and the unit of genetic distance was called the centi-Morgan (cM) in honour of Prof. T. H. Morgan. The Haldane or Kosambi mapping functions are commonly used to measure genetic distance. The Haldane mapping function takes into account the occurrence of multiple crossovers, whereas the Kosambi mapping function also considers interference caused by one crossing-over inhibiting the formation of another in its neighbourhood (Ott, 1985). Complete multipoint linkage analysis can be performed using computer programmes such as MAPMAKER (Lander *et al.*, 1987) or JOINMAP (Stam, 1993). The early linkage maps of barley were based on morphological and isozymes markers (reviewed by Von Wettstein-Knowles, 1992). Later, several barley genetic maps based on a variety of DNA markers (e.g. RFLP, RAPD, AFLPs, STSs, SSRs) were published (reviewed by Varshney *et al.*, 2004) and detailed information on most of these maps is available at the GrainGene website (http://wheat.pw.usda.gov/ggpages/maps.html). In addition to these maps, integrated genetic maps based on information from several independent DH progenies have been developed. These consensus maps are useful to determine marker locations on particular chromosomes and associations with other markers. When a genetic map is not available, mapping resistance genes can be achieved using Bulk Segregant Analysis (BSA), where DNA of non-segregating resistant and susceptible individuals from a segregating population are each pooled and screened for differences in the molecular markers (Michelmore *et al.*, 1991). Markers that are polymorphic between parents as well as bulks are then applied to the whole population and a partial map is constructed to locate the gene/s.

Seventeen of the 19 reported *Rph* genes have been assigned to a chromosome or a specific chromosome region (Table 2.6 and Fig. 2.4). With the help of molecular markers, 10 *Rph* genes have been located on barley chromosomes. Among the different molecular marker techniques, the RFLP method or STS markers derived from RFLP markers have been used frequently to locate leaf rust resistance genes in barley. This method has been used to map eight *Rph* genes. AFLP, RGA and SSR markers were used to map one resistance gene each, whereas, isozyme markers were used to map two resistance genes, *Rph10* and *Rph11* (Feuerstein *et al.*, 1990) (Table 2.6).

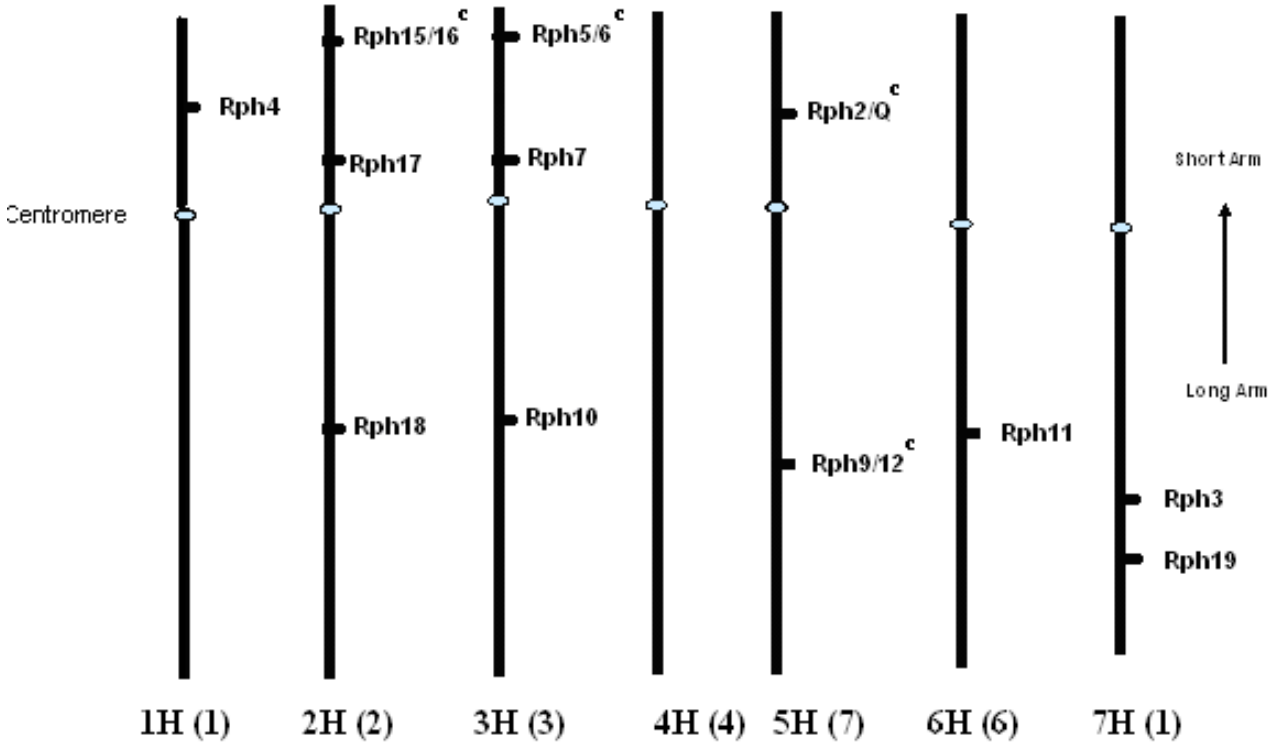
Table 2.6. Chromosomal location of *Rph* genes in barley.

Gene	Chromosome	Method of locus positioning	Closest marker	Reference
Rph1	2H	Trisomic analysis	-	Tuleen and McDaniel (1971); Tan (1978)
Rph2	5HS	Molecular marker (RFLP, STS)	CDO749, ITS1	Franckowiak *et al.* (1997); Borovkova *et al.* (1997)
Rph3	7HL	Morphological marker	-	Jin *et al.* (1993)
Rph4	1HS	Trisomic analysis, Molecular marker (RGA)	Probe 5.2	Tuleen and McDaniel (1971); Tan (1978) Collins *et al.* (2001)
Rph5	3HS	Trisomic analysis, Molecular marker (RFLP)	VT1	Tuleen and McDaniel (1971); Tan (1978) Mammadov *et al.* (2003)
Rph6	3HS	Molecular marker (RFLP)	BCD907, MWG2021	Zhong *et al.* (2003)
Rph7	3HS	Molecular marker (RFLP)	MWG691	Brunner *et al.* (2000); Graner *et al.* (2000)
Rph9	5HL	Molecular marker (STS)	ABC155, ABG3	Borovkova *et al.* (1998)
Rph10	3HL	Isozyme marker	-	Feuerstein *et al.* (1990)
Rph11	6HL	Isozyme marker	-	Feuerstein *et al.* (1990)
Rph12	5HL	Morphological marker, Molecular marker (STS, RAPD)	ABC155, OPA19	Jin *et al.* (1993); Borovkova *et al*. (1998)
Rph13	5HL	Linkage analysis with *Rph9* (30.4 ± 4.5 %)		Jin *et al.* (1996)

Rph15	2HS	Molecular marker (AFLP)	PM13M40	Weerasena *et al.* (2004)
Rph16	2HS	Molecular marker (RFLP, STS)	MWG874, MWG2133	Ivandic *et al.* (1998)
Rph17	2HS	Molecular marker (RFLP)	MWG682	Pickering *et al.* (1995); (1998)
Rph18	2HL	Molecular marker (RFLP)	MWG949	Pickering *et al.* (2000)
Rph19	7HL	Molecular marker (SSR)	HVM11	Park and Karakousis (2002)

† The chromosomal location of *Rph8* and *Rph14* have been not studied, ‡ *Rph5*/*Rph6* (Zhong *et al.*, 2003), *Rph9*/*Rph12* (Borovkova *et al*., 1998) and *Rph15*/*Rph16* (Weerasena *et al.*, 2004) were reported to be allelic

Figure 2.4. Chromosomal location[a] of seedling resistance genes against *P. hordei* in barley[b].



[a] Designation of barley chromosomes is based on the *Triticeae* system. The designation in the bracket is based on the original designation given by Burnham and Hagberg (1956).

[b] The chromosomal locations of *Rph8* and *Rph14* are not known. *Rph1* was located on 2H by trisomic analysis and *Rph13* is assumed to be on 5HL by linkage with *Rph9*.

[c] *Rph15*/16 (Weerasena *et al.*, 2004), *Rph5*/6 (Zhong *et al.*, 2003), *Rph2*/Q (Borovkova *et al.*, 1997), *Rph9*/12 (Borovkova *et al.*, 1998) are reported to be allelic.

2.9.3 Mapping quantitative traits

Characters exhibiting continuous variation are termed quantitative traits. Continuous variation can be caused by the simultaneous segregation of many genes, each producing a small phenotypic effect, and/or an effect of environment on the expression of the trait (Falconer and Mackay, 1996). Biometrical approaches have traditionally been used to analyse quantitative traits. QTLs can be mapped reliably using interval mapping based on maximum likelihood methods (Lander and Botstein, 1989) or multiple regressions (Haley and Knott, 1992). Interval mapping searches throughout a mapped genome for a single target QTL. The test is based upon the phenotypic means of the marker classes and the distance between the markers. Based on the interval mapping procedure, Lincoln *et al.* (1993) developed the computer programme MAPMAKER/QTL to analyse QTLs. In the presence of two or more QTLs per linkage group, this method may either fail to detect any effect at all, if the loci from a parent were of opposite sign, or may detect a ghost QTL (Martinez and Curnow, 1992). When multiple QTLs segregate, the sampling error associated with detection of a QTL may be inflated by the effects of other QTLs and linked QTLs can cause biased estimates of QTL position (Tinker and Mather, 1995). To overcome this problem, a composite interval mapping method that fits multiple QTLs was proposed (Jansen, 1993). The procedure involves the use of co-factors to account for variation in other regions of the genome when scanning a target region. Several computer software programmes such as MapQTL (Van Ooijen and Maliepaard, 1996), QTL Cartographer (Basten *et al.*, 1999) and PLABQTL (Utz and Melchinger, 1996) were designed to compute QTLs using the composite interval mapping procedure.

Table 2.7. Chromosomal locations of QTLs conferring resistance to *P. hordei* in barley

Population	**Chromosome**	**Closest marker**
Vada/L94	7H	E42M32-195 [a, b]
Vada/L94	2H	E41M32-83 [a, b]
Vada/L94	2H	E38M54-294 [a, b]
Vada/L94	4H	E35M61-368 [a, b]
Vada/L94	4H	E35M54-548 [b, c]
Vada/L94	6H	E37M33-574 [a, b, c]
Vada/L94	5H	E38M54-247 [a, b]
Vada/L94	5H	E33M61-595 [b]
Vada/L94	7H	E40M40-105 [b]
Vada/L94	7H	E40M32-123 [b]
Vada/L94	2H	E37M33-162 [b]
Vada/L94	2H	E38M54-134 [b]
Vada/L94	7H	E41M32-406 [b]
Harrington/TR306	5H	MG914 [d]
Harrington/TR306	2H	ABG058-WG516 [d]
Harrington/TR306	6H	WG223 [d]
Vada/IB-87	2H	S-217 [e]
Vada/IB-87	6H	m32p31-12 [e]

[a] Qi *et al.* (1998)

[b] Qi *et al.* (1999)

[c] Qi *et al.* (2000)

[d] Spaner *et al.* (1998)

[e] Backes *et al.* (2003)

Quantitative resistance against *P. hordei* was reported in the cultivar 'Vada' and the line 'TR306' (Table 2.7). Spaner *et al.* (1998) found three QTLs conferring resistance in a cross between 'Harrington' and the resistant line 'TR306'. These QTLs were located on 5H, 2H and 6H, and explained 45% of the total phenotypic variation. The partial resistance of 'Vada' and several other European cultivars was reported to be strongly correlated with long latent period, and genetic studies suggested that more than six genes contributed to long latent period in 'Vada' (Parlevliet, 1978). Qi *et al.* (1998) conducted a molecular analysis of partial resistance at seedling and adult plant growth stages using a high-density AFLP marker linkage map of a population derived from a cross between cultivar 'Vada' and the susceptible line 'L94'. This study also demonstrated six QTLs for long latent

period. Three QTLs, *Rphq1*, *Rphq2*, *Rphq3*, were effective at the seedling stage, while four QTLs, *Rphq2*, *Rphq3*, *Rphq4* and *Rphq5*, were effective at adult plant growth stages. Two QTLs (*Rphq2* and *Rphq3*) were consistently present at both seedling and adult plant growth stages. Race specificity for partial resistance was demonstrated by Qi *et al.* (1999). They identified an additional four QTLs for long latent period in cultivar 'Vada' when tests for long latent period were conducted using two pathotypes of *P. hordei*. Out of four QTLs, *Rphq7* was effective at the seedling stage, while *Rphq8*, *Rphq9* and *Rphq10* were effective at adult plant growth stages. An additional three QTLs were described from a cross between 'L94' and the partially resistant barley line '116-5', derived from a cross between 'Cebada Capa' and 'L94'. Only two QTLs, *Rphq2* and *Rphq3*, which were mapped to 2HL and on 6HS, were consistently effective in both studies at all growth stages against both races (Qi *et al.*, 1998; Qi *et al.*, 1999). Interestingly, molecular mapping using the population 'Vada'/'IB-87' identified only two QTLs responsible for resistance against *P. hordei* (Backes *et al.*, 2003), which were mapped on 2HL and 6H. In the course of this study, Backes *et al.* (2003) suggested a close relationship between the quantitative and qualitative types of resistance due to co-localisation of QTLs and resistance gene analogues (RGAs). This has been observed on a number of occasions in various host : pathogen relationships (Caranta *et al.*, 1997; Li *et al.*, 1999; Keller *et al.*, 1999; Geffroy *et al.*, 2000). RGAs were obtained by using conserved motifs from NBS-LRR type disease resistance genes, known to cause qualitative resistance against pathogens. Molecular mapping of several other QTLs in barley have resulted in them being localised on previously mapped qualitative resistance genes. This has been reported for powdery mildew (Backes *et al.*, 1996), net blotch disease (Richter *et al.*, 1998), stripe rust (Thomas *et al.*, 1995) and leaf rust (Thomas *et al.*, 1995; Kicherer *et al.*, 2000). These contrasting results on quantitative and qualitative resistance against pathogens warrant further analysis, and demonstrate the value of knowing the genotypes of host and pathogen in interpreting data applied to map based genetic analysis.

CHAPTER III

Seedling and adult plant resistance to *Puccinia hordei* in barley

3.1 Introduction

Leaf rust of barley, caused by *Puccina hordei,* is an economically important fungal disease in most temperate regions throughout the world including Australasia, Europe, North America and South America (Clifford, 1985). Significant yield losses (up to 32%) were reported due to leaf rust infection in susceptible barley cultivars in Australia and North America (Park and Karakousis, 2002). Deployment and utilisation of host genetic resistance is an economically and ecologically sustainable approach to control leaf rust in barley. To date, at least 19 *Rph* loci conferring seedling resistance to *P. hordei* have been characterised. Resistance provided by single *Rph* genes is frequently ephemeral and is often overcome by new pathotypes with matching virulence developing via mutation, introduction, selection or recombination (Park, 2003). Furthermore, it is known that pathotypes with virulence on genes *Rph1* to *Rph15* and *Rph19* are present in nature (Fetch *et al.*, 1998; Park and Karakousis, 2002). Therefore, alternate strategies including gene pyramiding and deployment of adult plant resistance (APR) were suggested as ways to increase the life of host resistance (Park, 2003). Several European barley cultivars, reputed for their slow leaf rusting character, were found to carry adequate resistance at adult plant growth stages to recent pathotypes of *P. hordei* under Australian conditions (Park, unpublished; Cotterill and Rees, 1993). APR is often an important component of durable resistance in wheat against wheat rust diseases (Roelfs, 1988, Singh and Rajaram, 1992, Barcellos *et al.*, 2000, Singh *et al.*, 2001). Finding novel sources of resistance in barley to *P. hordei* could allow the diversification of genetic resistance through breeding programmes. Therefore, the objective of the present study was to find new sources of resistance to leaf rust by screening diverse barley germplasm for the presence of seedling resistance and APR.

3.2 Materials and Methods

3.2.1 Plant material

The barley accessions examined comprised 87 commercial cultivars, two advanced breeding lines, two near isogenic lines, and a selection from an established cultivar. The original seeds of all genotypes were kindly provided by the Australian Winter Cereals Collection, Tamworth, NSW, Australia and the Plant Breeding Institute Cobbitty (PBIC) barley germplasm collection. The pedigree of each test genotype is given in Table 3.1. For greenhouse tests, seedlings of all genotypes and differential sets were raised in pots (9 cm diameter, containing pine bark fines and coarse sand) by sowing approximately 5 - 7 seeds of each line in a clump (two per pot). The pots were watered prior to sowing with a soluble fertiliser (Aquasol®, Hortico Pty. Ltd., Revesby, NSW, Australia) at the rate of 35 g per 3 L for 100 pots. To assess adult plant responses, 10 - 15 seeds of each line were hand sown in one meter rows at 0.5m spacing at two field sites (i.e. Karalee and Landsdowne) in 2006 in two replications. Rows of the susceptible cultivar 'Gus' were also sown after every five plots of test genotypes to allow uniform inoculum increase in the experimental areas. The experimental fields were irrigated as required and plots were fertilised at dough stage with Nitrofos ® at a rate of 20kg/hectare.

Table 3.1. Pedigrees of barley genotypes assessed for response to *Puccinia hordei* at seedling and adult plant growth stages.

Cultivar/Line	Accession no. [c]	Pedigree
Abacus (A)	400201	Vada*Zephyr
Abacus (B) [a]	400202	Unknown
Agio	400230	Kenia*Schweigers Georgine
Aladin	400011	(Abacus*Lud)*Armelle
Aramir	400284	Volla*Emir
Arrow	402910	(Lignee 39*Vada)*(Emir*Zephyr)
Atem	490045	((L 92*Minerva)*Emir)*Zephyr
Athos	BC	Lignee 207*Emir
Balder	400364	(Gull*Scanian barley)*Maja
Baronesse	BC	(Mentor*Minerva) * (Vada mutant*4* Carlsberg* Union) (Opavsky*Salle*3*Ricardo*5*Oriol*6153P40)
Belfor	400403	Minerva*(Heine 4808*Piroline)
Betina	491183	Vada Mutant (Dwarf)
Blenheim	402655	Triumph*Egmont
Casino	400024	((*H.deficiens**Sergeant)*Georgie)*Regent
Ceres	400583	Piroline*(Bordia*Kenia)
Cerise	400004	(Armelle*Lud)*Luke

Chariot	408125	Dera*(Carnival*Atem)
Claret	408173	((Proctor*HP 5466)*Armelle)*Abacus
Cornel	400658	Volla*(Emir*Cebeco 6010)
Corniche	400072	(Diamant*14029/64)*F2(Emir*(HOR 3270*46132/68))
Cygnet	BC	Target*Patty
Dash	BC	(Chad*Joline)*Cask
Delisa	400706	Delta*Wisa
Delta	400708	Tyra*Claret
Derkado	407510	Lada*Salome
Diva	407359	Volla*(Volla*Emir)
Draught	407577	Platoon*Chariot
Effendi	402843	Volla*(Wisa*Emir)
Efron	402984	Aramir*F1(Aramir*W 6165)
Egmont	402912	(Maris Yak*W 1001)*Vada
Emir	400780	Delta*(Agio*(Kenia)2*Arabian Variety)
Felicie	407183	Patty*Nadir
Fergie	405882	(Athos*Hood)*(Marion*Goldmarker)
Georgie	400884	Vada*Zephyr
Gilbert	BC	Koru reselection
Golf	400023	(Armelle*Lud)*Luke
Gull	400949	Gotland land cultivar
Hart	402733	Egmont*Atem
Hassan	400986	((Arabische*(Kenia)3)*Agio)*Delta
Havila	400006	Bomi*Aramir
Iban	402841	Aramir*LW 64192*(Zephyr*Sultan)
Javelin	402986	Athos*Trumpf
Julia	401146	Delta*Wisa
Kenia	401189	Binder*Gull
Klimek [a]	BC	Unknown
Lada	404731	St 49619/68*((Emir*St 11191/59)*Elgina)*St 46459/68*(Diamant*St 14008/64)
Lami	401257	Aura*Minerva
Landlord	407578	(Platoon*NFC86/60)*Chariot
Lina	405884	(Lofa Abed*3*Abed 6564)*(Mari 5*Multan)
Magnum	401325	Magnif 104*Universe
Menuet	404754	L 92*Minerva*Emir*Zephyr
Minerva	401434	*H.laevigatum**Gull
Miranda	402838	Volla*Vada
Mobek [a]	BC	Unknown
Monte Cristo	401473	Land cultivar, India
Nagrad	BC	RPB393173 X Georgie
Natasha	400082	Triumph*Aramir
Nomad	406002	(Armelle*Lud)*Luke
Nudinka	406806	Emir*L'Orge Nue de Weihenstephan
Optic	BC	Chad*(Corniche*Force)
Pallas	490001	Bonus X-Ray Mutant
P-10	490012	Pallas isogenic line for *Mla12*
P-23	490024	Pallas isogenic line for *MILa*
Patty	BC	Volla*Athos
Piroline	401747	Weihenstephaner Mehltauresistente CP*Morgenrot
Pirouette	401748	(Emir*(Heine 4808*Muller 61-223))*Delisa
Pompadour	BC	FDO192*Patty

Porthos	401762	207*Emir
RAH-1995 [a]	BC	Unknown
Rainbow	400026	Atem*Melody
Ramona	401814	Cambrinus*Emir
Regent	406433	Athos*Georgie
Ricardo	BC	Land cultivar
Roland	401864	Lud*Tellus M1D
Simba	401956	(Herta*BYG 191)*Minerva
Sundance	402027	Vada*Zephyr
Tintern	402083	(Sebarlis*(Zephyr)2*Emir)*(Zephyr)2
Toddy	407579	Optic*Chariot
Trinity	407398	Platton*Chariot
Tweed	403017	(Akka*Maris Mink)*Maris Mink
Tyne	402998	(Goldmarker*Athos)*(Goldmarker*Magnum)
Tyra	402149	(Algerian*Herta 8)*(Rika*Drost)
Ulandra (NT) [b]	BC	Selection Ulandra (Warboys*Alpha)
Union	402166	(Weihenstephaner Mehltauresistente 1*Donaria)*Firlbecks
Universe	402169	Abed 3371*Vada
Uta	402175	Emir*Quantum
Vada	BC	H.laevigatum*Gull
Varunda	402193	Vada*Hijlkema 1148
WI3407	BC	(Chieftain*Barque)*(Manley*VB9104)
Wisa	402259	Weihenstephaner Mehltauresistente 1*Isaria
Zita	406429	203/7748*Vada
Zulu	402301	(Triumph*Koru)*Goldmarker

[a] Pedigree information is not available
[b] Selection from Ulandra lacking *Rph2*
[c] BC – accessions were obtained from PBIC seed collection. All other numbers are accession numbers (AUS number) from the Australian Winter Cereal Collection at Tamworth, NSW

3.2.2 Pathogen material

Greenhouse inoculations were carried out using 10 Australian *P. hordei* pathotypes (*pts.*) (Table 3.2). Field inoculations were carried out using *pts*. 5653P+ (990492) and 5652P+ (010189) at the field sites Lansdowne and Karalee, respectively. The unique pathotype octal designations (Gilmour, 1973) are based on the virulence/avirulence pattern of an isolate on the standard differential genotypes, with the addition of P+ or P- to indicate virulence or avirulence on *Rph19*, respectively as suggested by Park (2003). All isolates originated from Australian pathogenicity surveys conducted from 1972 to 2001, and are maintained in cryogenic storage at the PBIC.

3.2.3 Inoculation methods and disease assessment

Greenhouse inoculations were carried out on 7 to 9-day-old seedlings with fully expanded first leaves. Urediniospores suspended in light mineral oil (Shellsol®, Mobil Oil, Sydney, Australia), at the rate of approximately 10 mg of spores per 10 mL oil per 200 pots, were atomised over seedlings in an enclosed chamber using a hydrocarbon propellant pressure pack. After each inoculation, the spray equipment was washed in 70% alcohol and rinsed in running tap water, and the enclosed chamber was spray washed with tap water for 5 min to avoid contamination between successive inoculations. Inoculated seedlings were incubated for 14-16 h at ambient temperatures in a misted dark room. The mist was generated by an ultrasonic humidifier. The seedlings were then moved to naturally lit greenhouse chambers at $20 \pm 2^{o}C$ and disease responses were scored after 10-12 days.

Field inoculations were performed following the technique outlined by McIntosh *et al.* (1995). To produce an epidemic in the field, a urediniospore-mineral oil suspension (mixing 30 mg of spores in 1.5 L of mineral oil) was misted over spreader rows using an ultra-low-volume applicator (Microfit®, Micron Sprayer Ltd., Bromyard, Herefordshire, UK). Four successive inoculations were carried out on afternoons when there was a high possibility of overnight dew. Random spots of 15 to 20 inoculated adult plants of spreader rows were subsequently sprinkled with water and covered overnight with plastic hoods to ensure adequate dew formation and infection in situations where natural dew formation was inadequate.

3.2.4 Scoring disease responses

Disease responses were scored in greenhouse tests using a 0-4 infection type (IT) scale (Park and Karakousis, 2002). Infection types of 3 or higher were regarded as indicating compatibility. Seedling resistance genes were postulated by comparing high and low IT patterns produced by different pathotypes on test cultivars with those of controls with known resistance genes.

Adult plant responses were recorded at regular weekly intervals, starting from the appearance of the first spikelet. A modified Cobb scale (Peterson *et al.*, 1948) was used to assess disease severity (percent leaf area affected) and host response (R, no uredinia present; Tr, traces or minute uredinia on leaf without sporulation; MR, small uredinia with slight sporulation; MR-MS, small to medium size uredinia with

moderate sporulation; medium size uredinia with moderate to heavy sporulation, S, large uredinia with abundant sporulation, uredinia often coalesced to form lesions).

A coefficient of infection (CI) was obtained from the disease severity and host response by multiplying the disease severity score by a predetermined value of 0.15, 0.30, 0.45, 0.60, 0.75 and 1.0 given to the host response ratings of R, MR, MR/MS, MS, MS/S and S, respectively. The rating of high, moderate and low APR was based on the average coefficient of infection (ACI) where ACI scores of 0-7, 8-14 and 15-22 were considered as having high, moderate and low APR. Cultivars with ACI values of 25 and above were regarded as lacking useful resistance and were included in the susceptible group.

3.3 Results

3.3.1 Seedling resistance

Seedlings of the 92 genotypes examined, along with 16 differential genotypes, displayed a range of infection types (ITs) when inoculated with a set of 10 *P. hordei* pathotypes. The seedling responses of differential genotypes are presented in Table 3.2. The array of ITs of the test genotypes was compared with those of differential genotypes to postulate the presence of known seedling resistance gene/s or uncharacterised resistance. Based on gene postulation results, the genotypes were categorised into nine groups.

Group 1: A total of 52 genotypes showed high ITs to all pathotypes and it was concluded that none carried detectable seedling resistance genes (Table 3.3). Whilst there was some evidence of incompatibility in some pathotype/host genotype combinations, this did not match any known resistance gene. Although ‘Athos’, ‘Patty’, ‘Pompadour’, ‘Draught’, ‘Trinity’ and ‘Varunda’ showed low ITs to certain pathotypes, these first tested results could not be repeated (data not presented).

Group 2: Twelve accessions (viz. ‘Blenheim’, ‘Corniche’, ‘Cygnet’, ‘Dash’, ‘Derkado’, ‘Javelin’, ‘Lada’, ‘Landlord’, ‘Mobek’, ‘Natasha’, ‘Optic’, and ‘Toddy’) showed low ITs with *pts*. 200P+, 211P+, 232P+, 242P+ and 253P-, and high ITs with *pts*. 4673P+, 5610P+, 5652P+, 5453P- and 5653P+ (Table 3.4).

Table 3.2. Infection types of differential genotypes to selected pathotypes of *Puccinia hordei*.

Differential genotype†	**Resistance gene/s**	**Octal Value**	**900233 200P+ [a]** [c]	**900021 211P+**	**920401 232P+**	**920636 243P+**	**760462 253P-**	**010187 4673P+**	**970073 5610P+**	**010037 5453P-**	**010189 5652P+**	**990492 5653P+ (+*Rph13*)**
Sudan	*Rph1*	1	1+NC	3+	2NC	3C	3+	3C	;1++NC	3+	;1N	3+
Peruvian	*Rph2*	2	;1+NC	1+NC	3+	3C	3C	3C	;1+C	3CN	3+	3+C
Estate	*Rph3*	4	;NC	;NC	0;N	;1NC	;1++	0;N	0;N	0;N	;1-N	;1CN
Gold	*Rph4*	10	3NC	3+	3+	;1NC	3+	3C	3C	3CN	3+	3+C
Magnif 104	*Rph5*	20	;1-N	0;N	3+	0;N	0;N	3	;N	0;N	0;N	0;N
Bolivia	*Rph2 + 6*	40	;1-NC	;1+NC	3	3+	3+	3+	;1-NC	3+	3+	3+
Cebada Capa	*Rph7*	100	0;N	0;N	0	0;N	0	0;N	0;N	0;N	0;N	0;N
Egypt 4	*Rph8*	200	3C	3+	3	3C	3C	3	3C	2+CN	3	3+
Abyssinian	*Rph9*	400	;1+N	1++NC	;1++N	;1N	;1-N	3+	3	3+	3+	3+
Clipper BC8	*Rph10*	1000	2+C	1++C	;1++C	2+C	;2+C	33+	33+	3+	3+	3
Clipper BC67	*Rph11*	2000	2N	2+N	2NC	2+N	1++2C	2++N	1++2N	2++N	2+NC	2+NC
Triumph	*Rph12*	4000	;1-NC	;1+N	;1-N	;1++NC	;1+NC	33+	33+	3C	3C	3CN
PI 531849	*Rph13*	10000	0;N	0;N	0;N	0;NC	0;N	0;N	0;N	0;N	0;N	3+
PI 584760	*Rph14*	20000	2+C	2N	;1N	1NC	2C	;1+CN	2+NC	1CN	;1++2CN	2CN
Prior	*Rph19*	P	3C	3+	3	3+	;1NC	3+	3+	1+CN	3+	3+
Cantala	*RphC* [b]	C	X 3	3C	X 1+C	3C	;1+NC	X 3C	33+	3+C	3	3CN

† Stocks carrying the gene *Rph15*, *Rph16*, *Rph17* and *Rph18* were not included

[a] Pathotype designations are as suggested by Park, 2003

[b] Uncharacterised resistance (R. F. Park, unpublished)

[c] Accession number

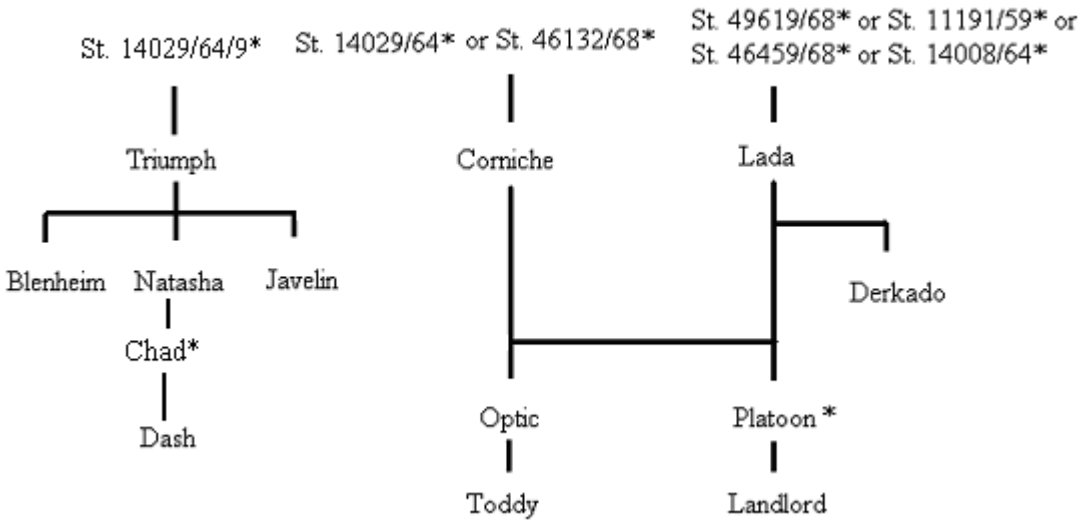
Table 3.3. Infection types of barley cultivars included in group 1 when inoculated with 10 *pts. Puccinia hordei*.

Cultivar/ Line	**200P+**	**211P+**	**232P+**	**243P+**	**253P-**	**5610P+**	**4673P+**	**5453P-**	**5652P+**	**5653P+**
Aramir	3+	3C	3	3+	3+	3CN	3	3+	3+	3C
Abacus (A)	3+	3	3+	3+	3	3+	3+	3CN	3N	3N
Agio	3+	3	3+	3+	3+	3+	3+	3+	3+	3+
Arrow	X 3	3N	3+	3	3+	3CN	3C	3CN	3	3C
Athos	3C	3CN	3C	3	3	3C	3CN	3	3CN	3CN
Balder	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Baronesse	3+	3+	3+	3+C	3C	3+	3	3+	3+	3C
Belfor	3+	3N	33+	3+	3+	3C	3C	3+	3	3
Betina	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Ceres	3+	3	3+	3+	3+	3+	3+	3+	3+	3
Cerise	3+	3C	3+	3+	3+	3+	3+	3	3+	3
Chariot	3	X 3C	3	X 3	3	3C	3C	33-	3	3CN
Cornel	3+	3C	3+	3N	3+	3CN	3CN	3CN	3	3CN
Delisa	3+	3+	3+	3+	3+	3+	3CN	3CN	3	3
Diva	3+	3	3+	3+	3+	3+C	3CN	3CN	3	3
Draught	3	3C	3+	3+	3+	3	3+	3CN	33+	3C
Effendi	3+	3C	3+	3	3+	3+	3	3CN	3+	3CN
Efron	3	3CN	3N	3CN	3N	3N	3	3CN	3N	3
Emir	3	3NC	3+	3+	3+	3NC	3C	3CN	3	3CN
Gilbert	3+	33+	33+	3	3	3+	3C	3+	3C	3+
Golf	3+	33+	3C	3	3+	3+	3+	3	3	3+
Gull	3+	3N	3+	3+	3+	3C	3+	3+	3	33+CN
Hassan	3+	3C	3	3+	3+	3C	3C	3CN	3	3CN
Havila	3	3+	3+	3	3+	3+	3+	3+	3+	3+

Julia	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Kenia	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Lami	3	3+	3	3	3+	3+	3+	3+	3	3+
Lina	33+	3+	3	3	3+	33+	33+	3+	33+	3CN
Minerva	3	3+	3+	3	3+	33+	3	3+	3+	3+
Miranda	3	X 3	3+	3	3+	3+	3C	3+	3	3C
Nagrad	3	3N	3+	3+	3+	3+	3+	3CN	3+	33+
Nomad	3	22+	3	3	33+	3	3C	3+	3	3
Nudinka	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Pallas	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
P-10	3+	3+	33+	3+	3+	3C	3+	33+	3+	3+
P-23	3+	3	3	33+	3+	3	3+	3C	3+	3+
Parthos	3+	3N	3+	3	3+	3C	3C	3CN	3	3CN
Patty	3+	3CN	3CN	3	3+	3	3+	3+	3CN	3C
Piroutte	3C	3C	3+	3+	3+	3	3+	3+	3+	3+
Pompadour	3	3CN	3	3	33+	3+	3+	3	33+	3C
RAH1990	3C	3N	3+	3CN	3C	3CN	3+	3CN	3	3CN
Ramona	3+	3C	3+	3+	3+	3+	3+	3+CN	3	3+
Simba	3	3	3	3	3	3C	3C	3CN	33-	3CN
Trinity	3	3	3	3	3	3	3N	3	3	3
Tweed	3+	3C	3	3C	3	33+	3C	3CN	3	3CN
Ulandra NT	3+	3N	3+	3+	3+	3+	3+	3CN	3C	3CN
Universe	3	3	3+	3+	33+	3+	3	3+	33+	3+
Uta	3	3	3+	3+	3+	3C	3CN	3CN	3	3CN
Vada	3	33+	3+	3	3	3+	3+	3	3+	3+
Varunda	3	3CN	3	3C	3CN	3	3+CN	3	3CN	3CN
WI 3407	3+	3	3+	3+	3+	3+	3+	3CN	3C	3C
Zulu	3	3+	3+	3+	3+	3+	3+	3+	3+	3+

This pattern of ITs was similar to that displayed by the differential genotypes carrying either *Rph12* or *Rph9* (Table 3.2). Therefore, all were postulated to carry either *Rph9* or *Rph12*. The possibility that these genotypes carried both *Rph9* and *Rph12* was excluded on the basis of previous studies that demonstrated they are allelic (Jin *et al*., 1996). Pedigree information suggested a common lineage between 11 of these genotypes that included the differential genotype for *Rph12*, Triumph (Fig. 3.1). Based on this information, they were postulated to carry *Rph12*.

Figure 3.1. Diagrammatic representation of genetic relationship between 10 cultivars and the source of resistance in them determined on the basis of pedigree information.



† The relationship of Cygnet and Mobek with other cultivars could not be identified by pedigree analysis.

* The genotypes were not included in the present study.

Group 3: The members of this group, **'**Atem', 'Georgie', 'Iban', 'Menuet', 'Regent' 'Sundance', 'Tintern', and 'Wisa', were postulated to carry *Rph*4. All showed low ITs with *pts*. 200P+ and 243P+ and high ITs to *pts.* 211P+, 232P+, 253P-, 4673P+, 5610P+, 5652P+, 5453P- and 5653P+, an IT pattern similar to the differential genotype 'Gold', known to carry *Rph4* (Table 3.5).

Table 3.4. Infection types of 12 barley genotypes included in group 2, postulated to carry *Rph12*, and the control differential genotype 'Triumph' when inoculated with 10 *pts*. of *Puccinia hordei*.

Cultivar	**200P+**	**211P+**	**232P+**	**243P+**	**253P-**	**4673P+**	**5610P+**	**5453P-**	**5652P+**	**5653P+**
Blenheim	;;CN	;1N	;N	2CN	;1-N	3	3	3+	3+	3+
Corniche	;1NC	1++N	0;N	;1N	;1N	3C	3C	3	33-	3C
Cygnet	;1-NC	1+N	;1-NC	;1-NC	;1++NC	3CN	3	3CN	3C	3CN
Dash	;1+N	1NC	;1-NC	;1-NC	;2NC	3C	3	3CN	33-	3CN
Derkado	;1CN	;1N	;1N	;1+N	;1-N	3	3	3+	3+	3+
Javelin	;1+N	;1+NC	2N	;1++2N	;1+N	33+	3+	3C	3+	3CN
Lada	;1-NC	;1NC	;1+NC	;1++N	;1-N	33+	3	3+	3	33+
Landlord	2+CN	;1+N	2+N	2+	2++N	3N	3N	3CN	3	3CN
Mobek	2N	;1++2C	;1CN	;12+C	2CN	3	3+	3+	3+	3+
Natasha	1NC	;1CN	;N	0;N	0;N	3CN	33+	3C	3C	3+C
Toddy	1NC	;1-NC	;1-NC	2NC	;2NC	3+C	3C	3+C	3	3C
Optic	;1+N	1+N	;1-NC	;1+NC	;2N	3C	3C	3C	3	3CN
Triumph	**;1-NC**	**;1+N**	**;1-N**	**;1++NC**	**;1+NC**	**33+**	**33+**	**3C**	**3C**	**3CN**

Table 3.5. Infection types of barley genotypes included in group 3, postulated to carry *Rph4*, and the control differential genotype 'Gold' when inoculated with 10 *pts. Puccinia hordei*.

Cultivar/ Line	**200P+**	**211P+**	**232P+**	**243P+**	**253P-**	**4673P+**	**5610P+**	**5453P-**	**5652P+**	**5653P+**
Atem	;2+C	33+	3+	;1NC	3	3	3CN	3+	3+	3
Georgie	;1++	3	3	;1++N	3	3C	3C	3CN	3	3CN
Iban	;N	3C	3+N	;N	3N	3+N	3N	3+N	3CN	3CN
Sundance	2+C	3	3	;1+NC	3C	3C	3+	3CN	3	3CN
Tintern	2+C	3N	3	;1++N	3	3+	3+	3CN	3	3CN
Menuet	1CN	33+	3+	;1-N	3+	3	3+	3+	3+	3+
Regent	2C	3CN	3	;2N	3N	3	2++N	33-N	3	3CN
Wisa	2+C	3	3	;1=CN	2++3CN	3+	3CN	3	3+	3
Gold	**3NC**	**3+**	**3+**	**;1NC**	**3+**	**3C**	**3C**	**3CN**	**3+**	**3+C**

Group 4: Eight genotypes ('Claret', 'Delta', 'Egmond', 'Hart', 'Ricardo', 'Tyra', 'Union' and 'Zita') displayed low ITs to *pts*. 200P+, 211P+ and 5610P+ and high ITs to *pts*. 232P+, 242P+, 253P-, 4673P+, 5652P+, 5453P- and 5653P+ (Table 3.6), the same pattern as observed for the differential genotype 'Peruvian'. All were therefore postulated to carry *Rph2*.

Group 5: 'Magnum' and 'Tyne' appeared to possess *Rph5* on the basis of their similar response to the *Rph5* differential 'Magnif 104'. All displayed low ITs to *pts*. 200P+, 211P+, 242P+, 253P-, 4673P+, 5610P+, 5652P+, 5453P- and 5653P+, and high ITs to *pts*. 232P+ and 4673P+ (Table 3.7).

Group 6: Low ITs were observed on 'Klimek' and 'Piroline' with *pts*. 232P+ and 253P- only (Table 3.8). These cultivars were therefore concluded to carry *RphCantala* because the array of ITs was similar to the Australian barley cultivar, 'Cantala'. This cultivar is known to carry an uncharacterised seedling resistance to *P. hordei* (Cotterill *et al*., 1994).

Group 7: A combination of *Rph2* and *Rph4* was postulated in cultivar 'Rainbow', on which low ITs were observed with *pts*. 200P+ (avirulent on *Rph2* and *Rph4*), 211P+, 5610P+ (avirulent on *Rph2*) and 243P+ (avirulent on *Rph4*), and high ITs with *pts*. 232P+, 253P-, 4673P+, 5652P+, 5453P-, and 5653P+ (all virulent on *Rph2*, and *Rph4*) (Table 3.9).

Group 8: The two cultivars 'Aladin', and 'Fergie' were postulated to carry a combination of *Rph1* with *Rph2*, because they showed high ITs with 243P+, 253P-, 4673P+, and 5453P+ (all virulent on *Rph1*, and *Rph2*), and low ITs with 211P+ (virulent on *Rph1*, and avirulent on *Rph2*), 232P+, 5652P+ (avirulent on *Rph1*, and virulent on *Rph2*), 200P+, and 5610P+ (avirulent on *Rph1*, and *Rph2*) (Table 3.10).

Group 9: Five genotypes 'Abacus (B)', 'Casino', 'Felicie', 'Monte Cristo' and 'Roland' expressed low ITs with all pathotypes (Table 3.11). The pathotypes used in this study were avirulent for *Rph3*, *Rph7*, *Rph11*, and *Rph14* (Table 3.2).

Table 3.6. Infection types of eight barley cultivars included in group 4, postulated to carry *Rph2*, and the control differential genotype 'Peruvian' when inoculated with10 *pts.* of *Puccinia hordei*.

Cultivar	**200P+**	**211P+**	**232P+**	**243P+**	**253P-**	**4673P+**	**5610P+**	**5453P-**	**5652P+**	**5653P+**
Claret	;CN	;1=N	3+	3+	3+	3+	;1-N	3N	3+	3+
Delta	;1+NC	;1-N	3	3+	3C	3C	0;N	3+	3+	3+
Egmont	;1++N	;1+NC	X 3	3C	3	3C	;1+NC	3+CN	3	3+
Hart	;N	;1=CN	3+	3	3+	3+	0;N	3	3+	3+
Ricardo [a]	1+CN	;1++N	3	3	3	3C	;1++CN	3	3CN	3CN
Tyra	1++CN	;1+CN	3	3	3	3+	;;1+N	3	3	3
Union	;1N	;1++N	3	3	3	3+	;1+N	3+	3+	3C
Zita	;1-NC	;1NC	33+	3	33+	3C	;1NC	3CN	3	3CN
Peruvian	**;1+NC**	**1+NC**	**3+**	**3C**	**3C**	**3C**	**;1+C**	**3CN**	**3+**	**3+C**

[a] Known to carry seedling resistance effective against *pts.* 5652P+, and 5653P+ (Park, R. F., unpublished)

Table 3.7. Infection types two of barley cultivars included in group 5, postulated to carry *Rph5*, and the differential genotype 'Magnif 104'when inoculated with 10 *pts.* of *Puccinia hordei*.

Cultivar	**200P+**	**211P+**	**232P+**	**243P+**	**253P-**	**4673P+**	**5610P+**	**5453P-**	**5652P+**	**5653P+**
Magnum	;CN	;N	3	;0N	0	3	0	0	0	0;N
Tyne	;N	;N	3+	;N	0	3+	0	;N	;N	;N
Magnif 104	**;1-N**	**0;N**	**3+**	**0;N**	**0;N**	**3**	**;N**	**0;N**	**0;N**	**0;N**

Table 3.8. Infection types of two barley cultivars included in group 6, postulated to carry *Rph Cantala*, and the differential genotype 'Cantala' when inoculated with 10 *pts.* of *Puccinia hordei*.

Cultivar	**200P+**	**211P+**	**232P+**	**243P+**	**253P-**	**4673P+**	**5610P+**	**5453P-**	**5652P+**	**5653P+**
Klimek	3+	3+	;1+NC	3C	1++2NC	3C	3+	3CN	3C	3CN
Piroline	3+	3+	2++C	3+	;1+N	3+	3+	3+	3+	3+
Cantala	**X 3**	**3C**	**X 1+C**	**3C**	**;1+NC**	**X 3C**	**33+**	**3+C**	**3**	**3CN**

Table 3.9. Infection types of barley cultivar Rainbow included in group 7, postulated to carry *Rph2*, and *Rph4*, and the control differential genotypes 'Peruvian', and 'Gold' when inoculated with 10 *pts.* of *Puccinia hordei*.

Cultivar	**200P+**	**211P+**	**232P+**	**243P+**	**253P-**	**4673P+**	**5610P+**	**5453P-**	**5652P+**	**5653P+**
Rainbow	;N	;N	3N	0;N	2+N	33+	0;N	3+	3+	3
Peruvian	**;1+NC**	**1+NC**	**3+**	**3C**	**3C**	**3C**	**;1+C**	**3CN**	**3+**	**3+C**
Gold	**3NC**	**3+**	**3+**	**;1NC**	**3+**	**3C**	**3C**	**3CN**	**3+**	**3+C**

Table 3.10. Infection types of two barley cultivars included in group 8, postulated to carry *Rph1*, and *Rph2*, and control differential genotypes 'Sudan', and 'Peruvian' when inoculated with 10 *pts.* of *Puccinia hordei*.

Cultivar	**200P+**	**211P+**	**232P+**	**243P+**	**253P-**	**4673P+**	**5610P+**	**5453P-**	**5652P+**	**5653P+**
Aladin	;CN	;1-N	0;N	;1+N	3	3+	;N	3C	0;N	3CN
Fergie	;1-CN	;1-CN	;N	2+CN	3CN	3	2+N	3CN	;1CN	3
Sudan	**1+NC**	**3+**	**2NC**	**3C**	**3+**	**3C**	**;1++NC**	**3+**	**;1N**	**3+**
Peruvian	**;1+NC**	**1+NC**	**3+**	**3C**	**3C**	**3C**	**;1+C**	**3CN**	**3+**	**3+C**

Table 3.11. Infection types of four barley cultivars included in group 9 when inoculated with 10 *pts.* of *Puccinia hordei*.

Cultivar/ Line	**200P+**	**211P+**	**232P+**	**243P+**	**253P-**	**4673P+**	**5610P+**	**5453P-**	**5652P+**	**5653P+**	**Probable genes**
Abacus (B)	;1CN	;2CN	;1+CN	;2+CN	;1+CN	;1+CN	;1+CN	;1+CN	;1+CN	;1+CN	?
Casino	2++3C	2CN	2++C	2++C	2++CN	2++CN	2N	2N	2CN	2CN	?
Felicie	;CN	;1=CN	2+CN	2++3CN	2+CN	2++3N	;1=N	;1+N	2CN	1++CN	?
Monte Cristo	;1CN	;1CN	;1CN	;1CN	;1CN	;1CN	;1CN	;1CN	;1CN	;1CN	?
Roland	;N	0	0	0	0	0	0;N	0;N	0	0;N	*Rph3* [a] or ?

[a] Based on pedigree information.

It is therefore possible that the genotypes possess one or more of these genes, or uncharacterised seedling resistance. The pedigree of these cultivars did not provide any information on the possible identity of the resistance present in them.

Two accessions of 'Abacus' (hereafter referred as 'Abacus (A)', and 'Abacus (B)', and included in group 1, and group 9, respectively) showed distinct ITs to the pathotypes (Plate 3.1). While 'Abacus (B)' was resistant to all pathotypes, 'Abacus (A)' was susceptible to all. The results demonstrated that accession 400202 ('Abacus' (B)') could be erroneous because the parents ('Vada' and 'Zephyr') of 'Abacus' lack seedling resistance that is effective to all the pathotypes.

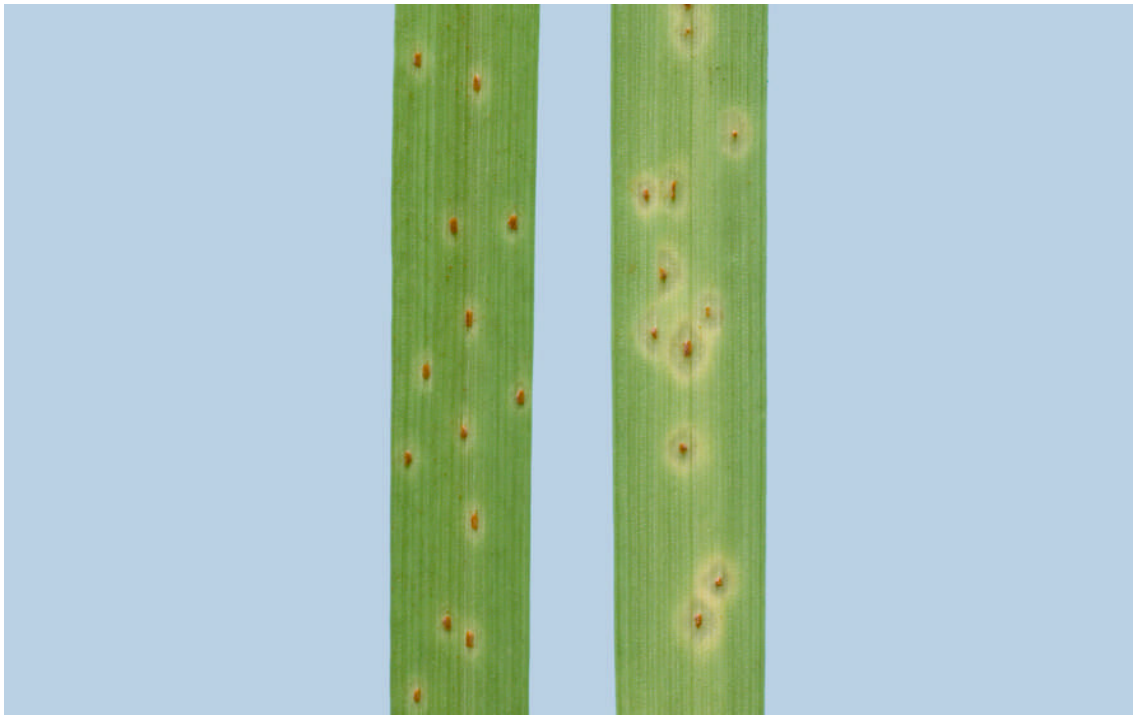


Plate 3.1. Infection types observed on seedlings (left to right) of 'Abacus (A)', and 'Abacus (B)' when inoculated with *P. hordei pt.* 5653P+.

3.3.2 Adult plant resistance

All genotypes were screened in replicated field trials in the 2006 cropping season at the PBI field sites Karalee and Lansdowne using *P. hordei pts.* 5652P+, and 5653P+, respectively. On the basis of adult plant responses, and results from seedling gene postulations, the cultivars were categorised into five groups.

Group A1: Fifty three genotypes displayed resistance at both field locations despite having no effective seedling resistance. Twenty of the 53 were postulated to carry *Rph12*, *Rph4*, *Rph2*, or *RphCantala* alone or in combination, whereas the remaining 33 genotypes lacked detectable seedling resistance (Table 3.12). Because the field pathotypes were virulent on these postulated seedling resistance genes, the resistance of these 53 genotypes observed in the field was concluded to be APR. Although 'Ricardo' was regarded as seedling susceptible, and therefore included in the APR category, previous studies have shown that it carries an uncharacterised seedling resistance effective to the field pathotypes. The failure to observe this seedling resistance in the present study could be because it displays environmental sensitivity (Park, R. F. unpublished).

The ACI values revealed that most of the 52 genotypes, notably 'Corniche' (0), 'Derkado' (0.08), 'Ricardo' (0.04), and 'Tweed' (0.08), displayed very high levels of APR. 'Egmont' (11.25), and 'Universe' (9.38) exhibited moderate levels of APR, whereas, low levels of APR were observed in 'Gilbert' (16.5), 'Atem' (18), 'Belfore' (18), 'Optic' (21.75), 'Uta' (20), and 'Klimek' (18.38) (Table 3.12).

Group A2: Twenty eight genotypes did not display effective resistance under field conditions, and were considered to be susceptible. Uredinia were large, and sporulation was profuse on all 28 but there were large differences in disease severity as evidenced by the large variation (26 to 75) in ACI values within the group. 'Betina' was the most susceptible cultivar, followed by susceptible check cultivar 'Gus', while 'P-23' (Pallas isogenic line for *MILa*) was the least susceptible (Table 3.13).

Group A3: Seven genotypes were resistant to the field pathotypes at the seedling stage as well as adult plant growth stages (Table 3.14). While 'Tyne', and 'Magnum' were postulated to carry *Rph5*, the identities of seedling resistance in 'Casino', 'Abacus', 'Felicie', 'Monte Cristo' and 'Roland' could not be determined with the pathotypes available. The resistance observed at adult plant growth stages for these genotypes was regarded to be due to the effectiveness of seedling resistance against the field pathotypes.

Table 3.12. Adult plant responses of barley genotypes included in group A1 to *Puccinia hordei* at two field sites in 2006.

	Landsdowne-2006 (*pt.* 5653P+)				**Karalee-2006 (*pt.* 5652P+)**				**ACI**	**Seedling**
Cultivar/Line	**Score**	**CI**	**Score**	**CI**	**Score**	**CI**	**Score**	**CI**		**resistance[a]**
Abacus (A)	TR	0.15	10MR	3	TR	0.15	TR	0.15	**0.86**	Nil
Agio	TR	0.15	TR	0.15	TR	0.15	TR	0.15	**0.15**	Nil
Aramir	5R	0.75	TR	0.15	TR	0.15	5R	0.75	**0.45**	Nil
Arrow	15MR	4.5	5R	0.75	5R	0.75	5R	0.75	**1.69**	Nil
Atem	40MS	24	30MS	18	20MS	12	30MS	18	**18**	*Rph4*
Athos	TR	0.15	TR	0.15	TR	0.15	TR	0.15	**0.15**	Nil
Baronesse	TR	0.15	5R	0.75	10R	1.5	10MR	3	**1.35**	Nil
Belfor	20MS	12	50MS	30	30MS	18	20MS	12	**18**	Nil
Chariot	TR	0.15	TR	0.15	TR	0.15	5R	0.75	**0.3**	Nil
Claret	5R	0.75	10MR	3	10/15MR	4.5	10MR	3	**2.81**	*Rph2*
Cornel	5R	0.75	TR	0.15	5R	0.75	5R	0.75	**0.6**	Nil
Corniche	0	0	0	0	0	0	0	0	**0**	*Rph12*
Cygnet	TR	0.15	0	0	5R	0.75	TR	0.15	**0.26**	*Rph12*
Dash	0	0	TR	0.15	TR	0.15	TR	0.15	**0.11**	*Rph12*
Derkado	0	0	TR	0.15	TR	0.15	0	0	**0.08**	*Rph12*
Draught	TR	0.15	TR	0.15	TR	0.15	TR	0.15	**0.15**	Nil
Egmont	20MS	12	20MS	12	20MS	12	15MS	9	**11.25**	*Rph2*
Emir	TR	0.15	TR	0.15	5R	0.75	TR	0.15	**0.3**	Nil
Georgie	TR	0.15	TR	0.15	TR	0.15	5R	0.75	**0.3**	Nil
Gilbert	30MS	18	30MS	18	20MS	12	30MS	18	**16.5**	Nil
Hart	10MR	3	5R	0.75	10MR	3	5R	0.75	**1.88**	*Rph2*
Hassan	TR	0.15	TR	0.15	TR	0.15	5R	0.75	**0.3**	Nil
Iban	10/15MR	4.5	20MR/MS	9	10MR	3	0	0	**4.13**	*Rph4*
Javelin	TR	0.15	TR	0.15	TR	0.15	0	0	**0.11**	*Rph12*
Klimek	40MS	24	30S	30	20MS	12	20MS/S	15	**18.38**	*RphCantala*
Lada	10MR	3	TR	0.15	10MR	3	10MR	3	**2.29**	*Rph12*

Landlord	TR	0.15	TR	0.15	5R	0.75	5R	0.75	**0.45**	*Rph12*
Miranda	5R	0.75	TR	0.15	10MR	3	5R	0.75	**1.16**	Nil
Minerva	10MR	3	10MR	3	5R	0.75	10/15MR	4.5	**2.81**	Nil
Nagrad	TR	0.15	TR	0.15	5/10R	1.5	5R/TR	0.75	**0.64**	Nil
Nomad	TR	0.15	TR	0.15	5/10R	1.5	10R	1.5	**0.83**	Nil
Optic	30MS/S	22.5	30/40S	30	30MS/S	22.5	20MS	12	**21.75**	*Rph12*
Patty	TR	0.15	TR	0.15	TR	0.15	TR	0.15	**0.15**	Nil
Pompadour	TR	0.15	TR	0.15	TR	0.15	TR	0.15	**0.15**	Nil
Porthos	TR	0.15	TR	0.15	TR	0.15	TR	0.15	**0.15**	Nil
RAH1995	TR	0.15	TR	0.15	5R	0.75	TR	0.15	**0.3**	Nil
Rainbow	10MR	3	10MR/MS	4.5	TR	0.15	5R	0.75	**2.10**	*Rph2 + Rph4*
Ramona	5R	0.75	TR	0.15	5R	0.75	TR	0.15	**0.45**	Nil
Regent	5R	0.75	TR	0.15	15MR/MS	6.75	0/TR	0.15	**1.95**	Nil
Ricardo	TR	0.15	0	0	0	0	0	0	**0.04**	*Rph2*+ ?
Simba	10MR	3	5R	0.75	10MR	3	0/TR	0.15	**1.73**	Nil
Sundance	TR	0.15	TR	0.15	TR	0.15	TR	0.15	**0.15**	*Rph4*
Tintern	TR	0.15	TR	0.15	TR	0.15	5R	0.75	**0.3**	*Rph4*
Toddy	TR	0.15	TR	0.15	TR	0.15	0/TR	0	**0.11**	*Rph12*
Trinity	TR	0.15	TR	0.15	TR	0.15	0/TR	0	**0.11**	Nil
Tweed	0	0	0	0	TR	0.15	TR	0.15	**0.08**	Nil
Ulandra (NT)	10MR	3	TR	0.15	5R	0.75	10MS	6	**2.48**	Nil
Universe	30MS	18	20MS	12	10/15MR	4.5	10MR	3	**9.38**	Nil
Uta	30MS/S	22.5	30MS/S	22.5	20MS/S	15	20S	20	**20**	Nil
Vada	TR	0.15	TR	0.15	TR	0.15	5R	0.75	**0.3**	Nil
Varunda	5R	0.75	TR	0.15	5R	0.75	5R/MR	1.5	**0.79**	Nil
WI 3407	5R	0.75	TR	0.15	TR	0.15	0	0	**0.26**	Nil
Zita	10MR	3	TR	0.15	TR	0.15	10MR	3	**1.58**	*Rph2*

[a] Results based on gene postulation experiments.

Table 3.13. Adult plant responses of barley genotypes included in group A2 to *Puccinia hordei* at two field sites in 2006.

	Landsdowne-2006 (*pt.* 5653P+)				Karalee-2006 (*pt.* 5652P+)				ACI	Seedling
Cultivar/Line	**Score**	**CI**	**Score**	**CI**	**Score**	**CI**	**Score**	**CI**		**resistance[a]**
Gus [b]	80S	80	70S	70	40S	40	50S	50	**60**	Nil
Balder	40S	40	40S	40	30S	30	40S	40	**37.5**	Nil
Betina	80S	80	90S	90	70S	70	60S	60	**75**	Nil
Blenheim	30S	30	30S	30	20S	20	20/30S	20	**25**	*Rph12*
Ceres	30MS/S	22.5	30S	30	40S	40	30S	30	**30.63**	Nil
Cerise	30S	30	30S	30	30S	30	30MS/S	22.5	**28.13**	Nil
Delisa	30MS/S	22.5	50/60S	50	20MS/S	15	10/15S	15	**25.63**	Nil
Delta	40MS/S	30	60S	60	20MS/S	15	20MS	12	**29.25**	*Rph2*
Diva	40S	40	60S	60	20S	20	30S	30	**37.5**	Nil
Effendi	40S	40	60S	60	30S	30	20S	20	**37.5**	Nil
Efron	30S	30	40S	40	20S	20	30MS/S	22.5	**28.13**	Nil
Golf	30S	30	40S	40	20S	20	20MS/S	15	**26.25**	Nil
Gull	30S	30	30S	30	20S	20	30S	30	**27.5**	Nil
Havila	20S	20	40S	40	30MS/S	22.5	30S	30	**28.13**	Nil
Julia	40S	40	30/40S	40	30S	30	20MS/S	15	**31.25**	Nil
Kenia	40MS	24	40S	40	30MS/S	22.5	30S	30	**31.63**	Nil
Lami	40MS	24	60/70S	70	20S	20	10S	10	**31**	Nil
Lina	40S	40	70S	70	30S	30	30S	30	**42.5**	Nil
Menuet	30/40S	40	30/40S	40	20S	20	30S	30	**32.5**	*Rph4*
Natasha	40/50S	50	50S	50	30S	30	30S	30	**40**	*Rph12*
Nudinka	30S	30	40S	40	20S	20	30S	30	**30**	Nil
Pallas	40S	40	70S	70	40S	40	30S	30	**45**	Nil
P-10	30MS/S	22.5	40MS	24	20S	20	20S	20	**31.63**	Nil
P-23	40MS	24	30S	30	30S	30	20S	20	**26**	Nil
Piroline	50S	50	60S	60	30MS/S	22.5	20S	20	**38.13**	*RphCantala*
Pirouette	60S	60	40/50S	50	20S	20	20S	20	**37.5**	Nil
Union	40S	40	40/50S	50	30S	30	20S	20	**35**	*Rph2*
Wisa	30MS/S	22.5	40MS/S	30	30S	30	40S	40	**30.63**	Nil
Zulu	30S	30	60S	60	20S	20	30S	30	**35**	Nil

[a] Results based on gene postulation experiments. [b] Control cultivar used as a susceptible spreader in the field experiments.

Therefore, the presence of potential APR underlying the seedling resistance could not be assessed. However, the high level of protection conferred by *Rph5*, and the uncharacterised seedling resistances in 'Casino', 'Abacus', 'Felicie', 'Monte Cristo' and 'Roland' at adult plant growth stages to field pathotypes was confirmed.

Group A4: 'Aladin', and 'Fergie', postulated to carry a combination of *Rph1* with *Rph2*, were included in this group. While, *Rph1* was effective against field *pt.* 5652P+, and ineffective against *pt.* 5653P+, *Rph2* was ineffective to both the pathotypes. Both cultivars exhibited high levels of resistance at both field sites (Table 3.15). The presence of APR against 5652P+ could not be determined because this pathotype was avirulent for the seedling resistance gene *Rph1*. However, the resistance displayed to *pt*. 5653P+ by both cultivars indicated the presence of APR underlying *Rph1* and *Rph2*.

Group A5: Two cultivars displayed adult plant responses that differed between pathotypes. 'Mobek' was resistant to *pt.* 5652P+ (CI = 3), and it was susceptible to *pt.* 5653P+ (CI = 30). 'Tyra' displayed high levels of APR against *pt.* 5653P+ (CI = 0.15), and was susceptible to *pt.* 5652P+ (CI = 30) (Table 3.16). The seedling resistance genes postulated in Tyra (*Rph2*), and Mobek (*Rph12*) could not explain the differences in resistance observed in the field because both genes were ineffective against the field pathotypes. Thus, these cultivars could have contrasting APR that showed evidence of pathotype specificity.

3.4 Discussion

The responses of 92 barley genotypes to *P. hordei* were assessed in the greenhouse at the seedling growth stage and in the field at adult plant growth stages to postulate the presence of seedling resistance genes and to identify potentially new sources of resistance. Pedigree information for each genotype was used to assist in interpreting the results.

3.4.1 Seedling resistance

The seedling response of the test cultivars to the array of *P. hordei* isolates demonstrated an absence of detectable seedling resistance in 52 genotypes and one or two *Rph* genes or uncharacterised resistance in the remaining 40 genotypes.

Table 3.14. Adult plant responses of barley genotypes included in group A3 to *Puccinia hordei* at two field sites in 2006.

	Landsdowne-2006 (*pt.* 5653P+)				Karalee-2006 (*pt.* 5652P+)				ACI	Seedling
Cultivar	**Score**	**CI**	**Score**	**CI**	**Score**	**CI**	**Score**	**CI**		**resistance[a]**
Abacus (B)	10MR	3	5R	0.75	TR	0.15	0	0	**0.98**	?
Casino	TR	0.15	TR	0.15	TR	0.15	TR	0.15	**0.15**	?
Felicie	0/TR	0.15	TR	0.15	TR	0.15	TR	0.15	**0.15**	?
Magnum	TR	0.15	TR	0.15	5R	0.75	5R	0.75	**0.45**	*Rph5*
Monte Cristo	TR	0.15	TR	0.15	TR	0.15	TR	0.15	**0.15**	?
Roland	TR	0.15	TR	0.15	TR	0.15	0/TR	0.15	**0.15**	*Rph3* or ?
Tyne	TR	0.15	TR	0.15	TR	0.15	0/TR	0.15	**0.15**	*Rph5*

[a] Results based on gene postulation experiments.

Table 3.15. Adult plant responses of barley genotypes included in group A4 to *Puccinia hordei* at two field sites in 2006.

	Landsdowne-2006 (*pt.* 5653P+)				Karalee-2006 (*pt.* 5652P+)				ACI	Seedling
Cultivar	**Score**	**CI**	**Score**	**CI**	**Score**	**CI**	**Score**	**CI**		**resistance[a]**
Aladin	TR	0.15	TR	0.15	0	0	TR	0.15	**0.11**	*Rph1 + Rph2*
Fergie	TR	0.15	TR	0.15	TR	0.15	0	0	**0.11**	*Rph1 + Rph2*

[a] Results based on gene postulation experiments.

Table 3.16. Adult plant responses of barley genotypes included in group A5 to *Puccinia hordei* at two field sites in 2006.

	Landsdowne-2006 (*pt.* 5653P+)				Karalee-2006 (*pt.* 5652P+)				ACI[b]	Seedling
Cultivar	**Score**	**CI**	**Score**	**CI**	**Score**	**CI**	**Score**	**CI**		**resistance[a]**
Tyra	50S	50	30S	30	TR	0.15	TR	0.15	-	*Rph2*
Mobek	TR	0.15	10MR	3	30S	30	30S	30	-	*Rph12*

[a] Results based on gene postulation experiment, [b] Not calculated due to effective APR against only one pathotype.

Twelve cultivars were postulated to carry either *Rph9*, and/or *Rph12*. These genes could not be discriminated because all Australian pathotypes of *P. hordei* are either virulent for *Rph9*, and *Rph12* or avirulent for both (Park, 2003). Although *Rph12* was originally characterised in the German cultivar 'Triumph' (also called 'Trumpf') (Walther, 1987; Jin *et al.*, 1993), one of the 'St. accessions', common in the pedigree of 'Triumph', 'Corniche', and 'Lada', was thought to be the original donor of *Rph12* (Dreiseitl and Steffenson, 2000). 'Triumph', 'Corniche', and 'Lada' or its derivatives were present in the pedigree of 'Blenheim', 'Natasha', 'Javelin', 'Dash', 'Optic', 'Toddy', 'Derkado', and 'Landlord', consistent with the presence of *Rph12* in all (Table 3.1, and Fig. 3.1).

Gene *Rph4* was identified in eight cultivars, six of which ('Atem', 'Georgie', 'Sundance', 'Tintern', 'Iban', and 'Menuet') were derived from the cultivar 'Zephyr'. Therefore, 'Zephyr' could be the donor of this gene in these cultivars. Cotterill *et al.* (1995) identified *Rph4* in 'Grimmett', a barley cultivar derived from 'Bussell' * 'Zephyr', and further reported that 'Bussell' lacked any seedling resistance gene, consistent with the hypothesis of 'Zephyr' as the donor source of *Rph4*.

'Georgie' and 'Atem' were present in the pedigrees of 'Regent' and 'Rainbow', respectively, supporting the postulation of *Rph4* in both. The cultivar 'Weihenstephaner Mehltauresistente', and 'Isaria' were reported previously to possess *Rph4* (Brückner, 1970) accounting for the detection of this gene in the cultivar 'Wisa' in the present study.

Eleven cultivars were postulated to carry *Rph2* alone or in combination with either *Rph4* or *Rph1*. Based on the responses of several barley cultivars with the *Rph2* gene to different isolates of *P. hordei* from North America, Africa, and Middle East, several researchers (Reinhold and Sharp, 1982; Steffenson and Jin, 1996; Tan, 1977b; Yahyaoui and Sharp, 1987) suggested that an allelic series exists at the *Rph2* locus. In the present experiments, all genotypes postulated to carry *Rph2*, were believed to carry the 'Peruvian' allele, as indicated by the similar responses of the genotypes and 'Peruvian' to different pathotypes of *P. hordei* (Table 3.6, 3.9, 3.10). 'Claret', 'Aladin', and one of the parental genotypes of 'Fergie' (Hood) were derived from 'Armelle', which was previously postulated to possess *Rph2* (Parlevliet, 1983).

In addition to *Rph2*, 'Aladin' and 'Fergie' were also thought to carry *Rph1* (Table 3.10). The source of *Rph1* in these cultivars could not be determined on the basis of available pedigree information. The postulation of *Rph2* in 'Union', and 'Tyra' was supported by similar postulations by Brückner (1970), and Parlevliet (1983), respectively. The donor of *Rph2* in 'Egmont' could be either 'Maris Yak' or 'W 1001', and the gene in 'Zita' could have been derived from the line '203/7748'. This conclusion was drawn because 'Vada', a common parental genotype of both cultivars, lacked detectable seedling resistance. 'Delta', derived from the cross between 'Tyra'*'Claret', and 'Hart' developed from 'Egmont', were also postulated to carry *Rph2*. 'Ricardo' is in the Australian sub set of differential genotypes used to characterise pathotypes of *P. hordei*, and is believed to carry *Rph2* plus an uncharacterised seedling resistance (R. F. Park, unpublished). Another uncharacterised seedling resistance gene, present in 'Cantala' (referred as *RphCantala*, Park, 2003), was postulated in the Polish cultivar 'Mobek', and in 'Piroline'. 'Cantala' was derived from a cross between 'Erectoides 16' (a semidwarf mutant of 'Maja'), and 'Kenia'. Based on the gene postulation results of the present study, it was concluded that the resistance gene in 'Cantala' might have been obtained from 'Erectoides 16' because the second parent 'Kenia', lacked detectable seedling genes. The donor source of the gene in 'Mobek' could not be verified because pedigree information was not available for this cultivar. 'Tyne' was derived from 'Magnum', and both the cultivars were predicted to carry *Rph5*. The presence of 'Magnif 104', a differential genotype for *Rph5,* in the pedigree of 'Magnum' supports the prediction of this gene in these cultivars.

The seedling resistance displayed by 'Abacus (B)', 'Casino', 'Felicie', Monte Cristo' and 'Roland' could not be explained with the array of pathotypes used, and all were therefore concluded to carry uncharacterised resistance. However, 'Roland' was presumed to carry *Rph3*. 'Meltan', derived from 'Tellus MHM DDN' * ('Triumph'*'Georgie') was postulated to carry *Rph3* (Niks *et al.*, 2000). In the present study, 'Triumph', and 'Georgie' were postulated carry *Rph12* and *Rph4*, respectively. The results indicates that 'Tellus MHM DDN', present in the pedigree of 'Roland', could be the source of *Rph3* in 'Meltan'.

The known seedling resistance genes *Rph1*, *Rph2*, *Rph4*, *Rph5*, *Rph12* and *RphCantala*, identified in the present study, are not useful in Australia because

virulence for all these genes have been detected in barley growing regions of Australia (Park, 2003). Widespread occurrence of virulence on many of these seedling genes has also been reported in other parts of world, although *Rph5* remains effective in North America (Mammadov *et al.*, 2003). The unknown seedling resistances present in 'Abacus (B)', 'Casino', 'Felicie', and Monte Cristo 'and 'Roland' was effective to all Australian pathotypes of *P. hordei*, indicating that they could be potential sources for leaf rust resistance. Genetic studies are needed to characterise these resistances.

3.4.2 Adult plant resistance

Field assessments of genotypes that lacked seedling resistance genes or that carried seedling resistance ineffective to the field pathotypes used revealed the presence of APR in 53 genotypes, whereas 28 genotypes were classified as lacking effective APR. The APR in nine cultivars could not be assessed because all possessed seedling resistance genes that were effective against one or both of the field pathotypes. Two cultivars carried APR that was effective against only one field pathotype.

Pedigree information was used to ascertain the possible genetic relatedness of the genotypes identified as carrying APR (Fig. 3.2). This analysis along with the field results, suggested two possible origins of APR in the test genotypes. 'Vada', and 'Minerva', obtained from a cross between '*H. laevigatum*', and 'Gull' (Dros, 1957) were considered to be the source of APR in 26 cultivars. The APR in Vada and Minerva may have originated from '*H. laevigatum*' because the second parent, 'Gull' was susceptible to the field pathotypes (Plate 3.2 and Fig. 3.2).

'Emir', derived from the cross 'Delta'*'Agio'*'Kenia'*'Arabian cultivar', was considered to be the source of APR in 18 cultivars (viz. 'Athos', 'Aramir', 'Cornel', 'Corniche', 'Cygnet', 'Derkado', 'Fergie', 'Iban', 'Javelin', 'Lada', 'Optic', 'Porthos', 'Patty', 'Pompadour', 'Ramona', 'Tintern', 'Tweed', and 'Uta'). A lack of effective APR in 'Delta', and 'Kenia' implied that the APR in 'Emir' could have been derived from 'Agio', and/or 'Arabian cultivar' (Plate 3.3 and Fig. 3.2). The donor of APR in four (viz. 'Arrow', 'Optic', 'Regent', and 'Toddy') cultivars could be either 'Vada', and/or 'Emir' because they were derived using both of these sources. The original donor sources of the resistance in four genotypes (viz.

'Ricardo', 'WI3407', 'RAH1995', 'Ulandra (NT)') could not be suggested based on pedigree information (Plate 3.4).

Seven cultivars derived from 'Vada', and 'Minerva' ('Cerise', 'Zulu', 'Golf', 'Blenheim', 'Menuet', 'Betina', and 'Lami'), and nine cultivars derived from 'Emir' ('Havila', 'Natasha', 'Effendi', 'Nudinka', 'Diva', 'Piroutte', 'Delta', and 'Delisa') displayed large uredinia with abundant sporulation, and lacked effective APR (Fig. 3.2). However the disease severity on all was less than the susceptible check, 'Gus' (Table 3.13). The differences in disease severity could be because of environmental factors, and/or the growth stages of the cultivars at the time of disease scoring. However, the possible presence of minor gene/s in these genotypes cannot be excluded.

Plate 3.2. Adult plant (flag leaf) responses (left to right) of 'Vada', 'Minerva', 'Gull', and 'Gus' when assessed under field conditions against *P. hordei pt.* 5653P+.

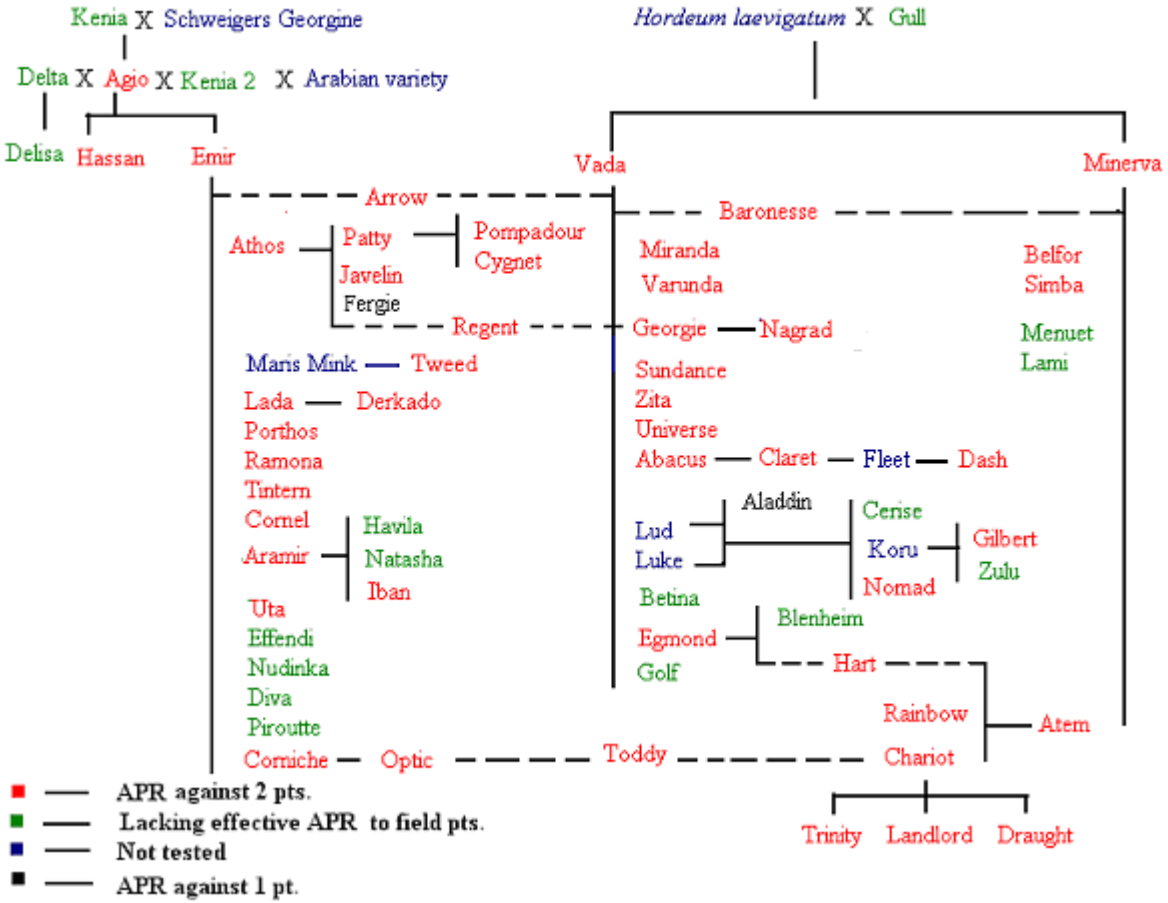


Figure 3.2. Diagrammatic representation of genetic relationships between barley genotypes based on pedigree information, and the presumed origin of APR based on their responses to *Puccinia hordei* at adult plant growth stages. Letters with red, black, green, and blue colour denote the presence of APR against both pathotypes, presence of APR against 1 pathotype, absence of useful APR, and genotypes not assessed in this experiment.

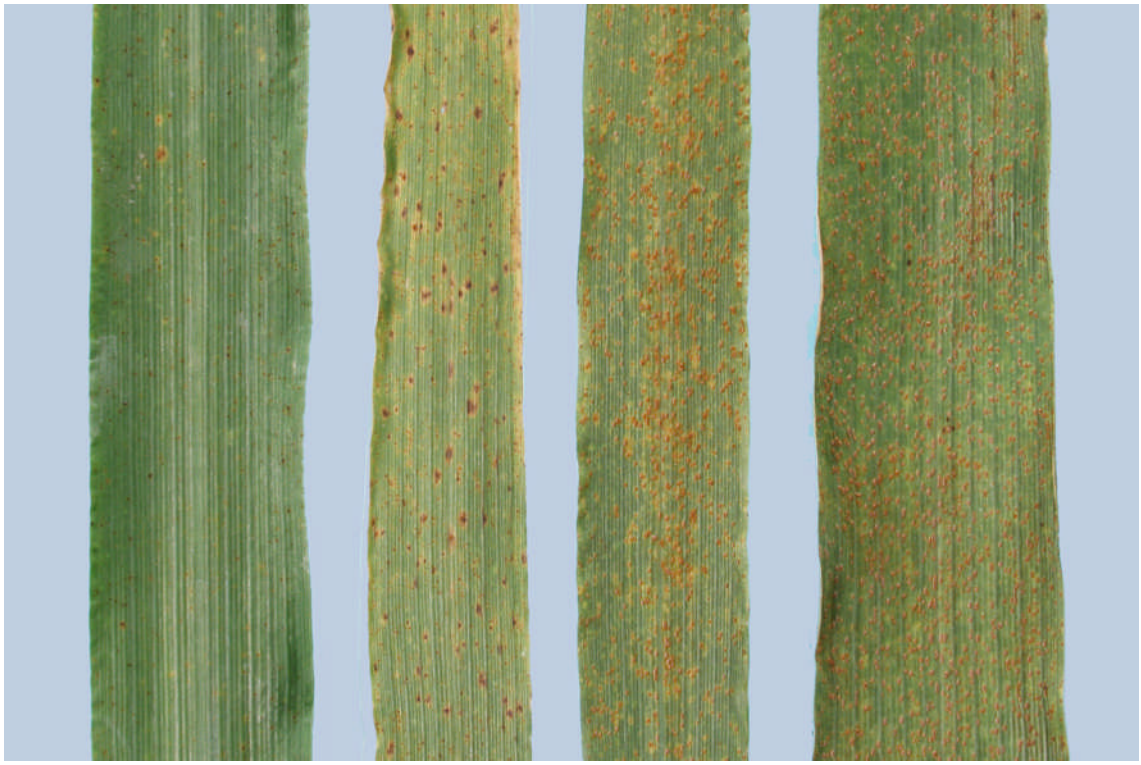


Plate 3.3. Adult plant (flag leaf) responses of 'Emir', 'Agio', 'Delta', and 'Kenia' when assessed under field conditions against *P. hordei pt.* 5653P+.

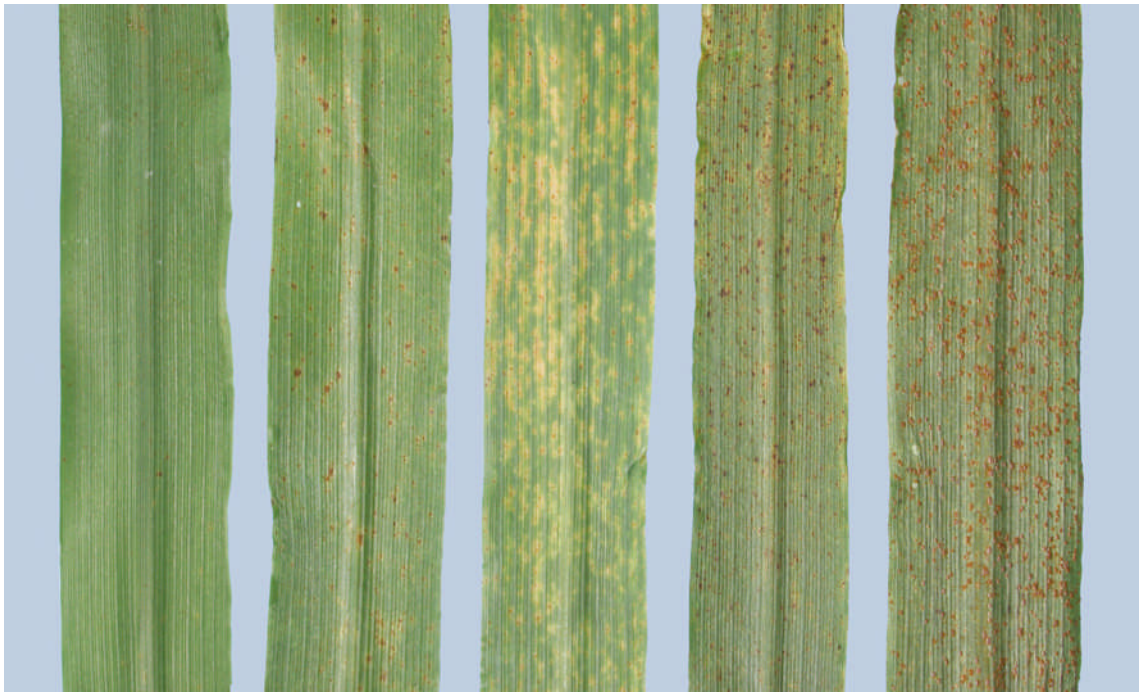


Plate 3.4. Adult plant (flag leaf) responses (left to right) of 'Ricardo', 'WI3407', 'RAH1995', 'Ulandra (NT)', and 'Gus' infected with *P. hordei pt.* 5653P+.

It is of interest to note that the presumed donor sources of APR, 'Vada', 'Minerva', and 'Emir', were also reported previously to possess partial resistance to *P. hordei* (Parlevliet, 1979, 1983). This type of resistance was also shown to be present in many West-European cultivars (Parlevliet *et al.*, 1980), most of which were derived from 'Vada', 'Minerva', and/or 'Emir'. Jensen and Jørgensen (1991) believed that the presence of partial resistance in these cultivars could be because of the linkage of powdery mildew resistance gene, *MILa* derived from *H. laevigatum*, and one of the genes responsible for partial resistance to leaf rust. In the present study, 'Pallas', and one of its isogenic lines for *MILa* ('P-23') were susceptible at seedling as well as adult plant growth stages, indicating a lack of association between the APR to leaf rust identified and the *laevigatum* powdery mildew resistance gene. Although the line was classed as susceptible, it gave consistently low ACI value, indicating a possible minor effect of the *MILa* region on leaf rust resistance (Table 3.13).

Partial resistance is characterised by a reduced rate of epidemic development despite a susceptible infection type at all growth stages, and was considered distinct from seedling, and adult plant resistance (Parlevliet and Ommeren, 1975). In the present study, the responses of several cultivars reported previously to carry partial resistance clearly carried APR. The expression of partial resistance was higher at adult plant growth stages (Parlevliet and Ommeren, 1975), as supported by histological studies of resistance in the barley cultivar 'Vada' that showed early abortion of hyphal growth at adult plant growth stages, in contrast to seedling growth stages (Parlevliet and Kievit, 1986). Cultivars with partial resistance differ greatly for parameters such as infection frequency, latent period, rate of spore production, and period of spore production. Among these parameters, latent period was found to be highly correlated with partial resistance, and it was used to evaluate the level of partial resistance in subsequent studies (Parlevliet, 1979). Latent period was further described to be pleiotropically associated with infection frequency at adult plant growth stages (Parlevliet, 1986). This was observed in the responses of many cultivars in the present experiment. For example, 'Porthos', 'Georgie', 'Varunda', 'Hassan', and 'Ramona', with significantly higher partial resistance (Parlevliet *et al.*, 1980), were also found to carry high levels of APR (ACI less than 1.0) (Table 3.12). Consequently, it is tempting to say that the APR to *P. hordei* observed in the present

study may be associated with, or even the same, as the partial resistance reported in previous studies. Furthermore, the results obtained by Parlevliet and Kievit (1986), demonstrating the importance of growth stage on the expression of resistance to *P. hordei*, reveals that partial resistance could also be evaluated at adult plant growth stages using a parameter like infection frequency. The scale used in the present study to assess disease under field conditions was used extensively in the identification, characterisation, and deployment of a number of APR genes in wheat to wheat rust diseases (McIntosh *et al.*, 1995). The potential association of APR identified in several other cultivars such as 'Cornel', 'Patty', 'Pompadour', 'Athos', 'Nagrad', 'Baronesse', 'WI3407', 'RAH1995', 'Gilbert', 'Dash' and 'Derkado' with partial resistance is not known.

In conclusion, barley genotypes were assessed for leaf rust response at seedling and adult plant growth stages, and together with pedigree information, postulation of known, and unknown resistance to *P. hordei*, and the donor sources of the resistance were made. Fifty seven barley genotypes were concluded to carry APR effective to current Australian pathotypes of *P. hordei*. Because the APR sources identified in the present study provided adequate levels of protection, genetic analyses of resistance in these genotypes are needed for their efficient use in breeding for leaf rust resistance. The seedling resistances identified in 'Abacus (B)', 'Casino', 'Felicie', 'Monte Cristo' and 'Roland' were also shown to confer very high levels of protection against *P. hordei* pathotypes at adult plant growth stages, and they could be potentially useful sources of resistances if the number of genes governing the resistance in each genotype, and their relationships with other known resistance genes are established.

CHAPTER IV

Genetic analysis of adult plant resistance to *Puccinia hordei* in barley

4.1 Introduction

Resistance to leaf rust in barley (caused by *Puccinia hordei*) has been categorised broadly into seedling resistance and partial resistance. Seedling resistance is usually governed by single genes that are expressed at all growth stages, and it is relatively easy to identify and incorporate into elite germplasm. To date, several seedling genes conferring resistance to leaf rust in barley have been described, of which 19 are designated *Rph1* to *Rph19* (Weerasena *et al*., 2004). Cultivars carrying these *Rph* genes have been released in many parts of the world, including Europe, North America, Australia and New Zealand (Park, 2003; Weerasena *et al*., 2004; Ye *et al*., 2007). However, the seedling resistance conferred by these *Rph* genes has been frequently overcome by pathotypes with matching virulence (Park, 2003). In contrast to seedling resistance, partial resistance is difficult to utilise in barley breeding programmes due to its low levels of expression and quantitative inheritance (Parlevliet and Kuiper, 1985). Another form of resistance that expresses only during post-seedling growth stages, adult plant resistance (APR), has been well characterised and utilised in wheat to control rust diseases (McIntosh *et al.*, 1995). This form of resistance in wheat has often provided durable resistance against leaf rust (*Lr34* and *Lr46*), stem rust (*Sr2*), and stripe rust (*Yr18* and *Yr29*) despite being monogenic. Because of the value of APR to rust in wheat, it was considered worthwhile to investigate APR to leaf rust in barley as a potential strategy for successful long term disease management.

In a previous study, several barley genotypes were found to carry good levels of APR to current pathotypes of *P. hordei* (R. F. Park, unpublished; Golegaonkar *et al.*, 2006). Based on parentage, the APR identified in these genotypes was concluded to have originated from at least three sources. The objective of the present study was to gain a better understanding of the APR identified previously by undertaking genetic analyses on 10 barley genotypes to determine the number of genes governing the resistance in each, and the genetic relationships between them.

4.2 Materials and Methods

4.2.1 Plant material and field design

The pedigrees and countries of origin of the 10 barley genotypes used as resistant parents are given in Table 4.1. Previous studies established that 'Ulandra' was heterogeneous for the presence of *Rph2,* and selections carrying *Rph2* ('Ulandra (T)'), and lacking *Rph2* ('Ulandra (NT)') were established (R. F. Park, unpublished). To develop BC_1F_2 populations for genetic analyses of APR, six barley cultivars, two advanced breeding lines and 'Ulandra (NT)' (all two row types) were hybridised with the leaf rust susceptible spring barley cultivar 'Gus' (six row type) as a common recurrent male parent. All parents and BC_1F_2 seeds were space planted (approximately 25 to 30 seeds/line in 2m rows) in rust nurseries at two field sites (Lansdowne and Karalee) at the Plant Breeding Institute Cobbitty (PBIC), Australia. The resistant genotypes 'Patty', 'Pompadour', 'Nagrad', 'RAH1995', 'Athos', 'Gilbert' and 'WI3407' were crossed with 'Vada' to generate F_2 populations in tests of allelism. Based on the results of the cross 'Vada'/ 'WI3407', 'WI3407' was further crossed with 'Patty', 'Pompadour', 'Nagrad' and 'RAH1995' to generate additional F_2 populations. Approximately 150 to 250 F_2 seeds from each R X R cross were field planted 15cm apart in 10m long rows to assess adult plant response to *P. hordei*. Rows of the susceptible cultivar 'Gus' were also sown surrounding the experimental area and after every five plots to allow inoculum increase.

4.2.2 Pathogen

Field inoculations were carried out using *pts*. 5653P+ and 5453P- at Karalee and Landsdowne sites of PBIC, respectively. Details of pathotype nomenclature are provided in Chapter 3. All 10 cultivars examined were seedling susceptible to these pathotypes. To induce an artificial leaf rust epidemic in the field, a urediniospore-mineral oil suspension (mixing 30 mg of spores in 1.5 L of mineral oil) of each pathotype was misted over spreader rows using an ultra-low-volume applicator (Microfit®, Micron Sprayer Ltd., Bromyard, Herefordshire, UK). Four successive inoculations were carried out on afternoons when there was a high likelihood of overnight dew. On each occasion, random spots (15 to 20) within the inoculated spreader rows were subsequently sprinkled with water and covered overnight with plastic hoods to ensure dew deposition and infection in case adequate natural dew formation did not occur. The identities of prevailing field pathotypes that became

established at each field site were confirmed after the onset of epidemics by testing 10 random samples collected from each field site following the pathogenicity method described by Park (2003).

4.2.3 Disease assessment

Adult plant responses were assessed at least three times following anthesis, when disease severity on the susceptible check was 60S or higher. While the parents and F_2 plants were scored as either resistant or susceptible, BC_1F_2 rows were scored as either non-segregating susceptible or segregating. A modified Cobb scale (Peterson *et al.*, 1948) was used to assess disease severity (percent leaf area affected) and host response (R, no uredinia present; Tr, trace or minute uredinia on leaves without sporulation; MR, small uredinia with slight sporulation; MR-MS, small to medium size uredinia with moderate sporulation; MS-S, medium size uredinia with moderate to heavy sporulation; S, large uredinia with abundant sporulation, uredinia often coalesced to form lesions).

4.2.4 Statistical analysis

The field data obtained from BC_1F_2 rows (R X S crosses) and F_2 plants (R X R crosses) were subjected to Chi-squared ($\chi 2$) analysis to confirm the goodness-of-fit of observed ratios to theoretical expectations.

Table 4.1. Pedigrees and countries of origin of resistant parental barley genotypes

Cultivar/line	**Pedigree**	**Country of origin**
Athos	Lignee 207*Emir	France
Dash	(Chad*Joline)*Cask	UK
Gilbert	Reselection from Koru	Australia
Nagrad	RPB393173*Georgie	Polish
Patty	Volla*Athos	France
Pompadour	FDO192*Patty	France
RAH 1995 [a]	Unknown	Poland
Ulandra (NT)	Selection from Ulandra lacking *Rph2*	Australia
Vada	*H. laevigatum**Gull	Netherlands
WI3407 [a]	(Chieftain*Barque)*(Manley*VB9104)	Australia

[a] advanced breeding line

4.3 Results

The genetic analyses of APR in 10 barley genotypes were carried by screening the BC_1F_2 populations under an artificial epidemic of *P. hordei* at two field sites in 2005 and 2006. Very good leaf rust epidemics developed at the field sites in both years.

The results of multipathotype tests and field observations of 'Athos', 'Dash', 'Gilbert', 'Patty', 'Pompadour', 'Nagrad', 'RAH1995', 'Ulandra (NT)', 'Vada' and 'WI3407' demonstrated APR to *P. hordei* in all (Table 4.2). In the field tests, cultivar 'Gilbert' developed disease responses of up to 30MS under high disease pressure, whereas all other genotypes were consistently rated TR to 10 MR (Table 4.2). Excessive necrotic flecks with or without chlorosis were common on the flag leaves of all the resistant parents, which were clearly distinguishable from the susceptible cultivar 'Gus' (Plates 4.1 and 4.2).

Table 4.2. Parental reactions to *Puccinia hordei* at seedling (greenhouse) and adult plant (field) growth stages

Cultivar/line	**Seedling response** [a]			**Adult plant response** [b]	
	5652P+	**5653P+**	**Postulated seedling resistance** [c]	**Karalee** [d]	**Lansdowne** [e]
Athos	3CN	3CN	Nil	TR	TR
Dash	33-	3CN	*Rph12*	0 to TR	0 to TR
Gilbert	3C	3+	Nil	10 MR	15 MR to 30MS
Nagrad	3+	33+	Nil	TR	TR
Patty	3CN	3C	Nil	TR	TR
Pompadour	33+	3C	Nil	TR	TR
RAH1995	33+	3+	Nil	TR	TR
Ulandra (NT)	3	3C	Nil	TR	TR to10MR
Vada	3+	3+	Nil	TR	TR to 10MR
WI3407	3C	3C	Nil	TR	TR to 10MR
Gus	3+	3+	Nil	60S	80S

[a] 0 to 4 infection scale (Park *et al.*, 2003), where scores of 3 and higher were considered disease compatible
[b] Indicates responses observed over three cropping years (2004, 2005 and 2006)
[c] Based on multipathotype tests using 17 pathotypes of *P. hordei* (Golegaonkar *et al.*, 2006)
[d] Evaluated against *pt*. 5453P- in 2004, 2005 and *pt*.5652P+ in 2006
[e] Evaluated against *pt*.5652P+ in 2004, 2005 and *pt*. 5653P+ in 2006

Plate 4.1. Adult plant flag leaf rust responses of (from left) ‘Gus’ (susceptible, male parent), ‘Pompadour’, ‘Patty’, ‘Nagrad’, ‘Vada’, ‘RAH1995’ and ‘WI3407’ when tested in the field against *Puccinia hordei* pathotype 5653P+.

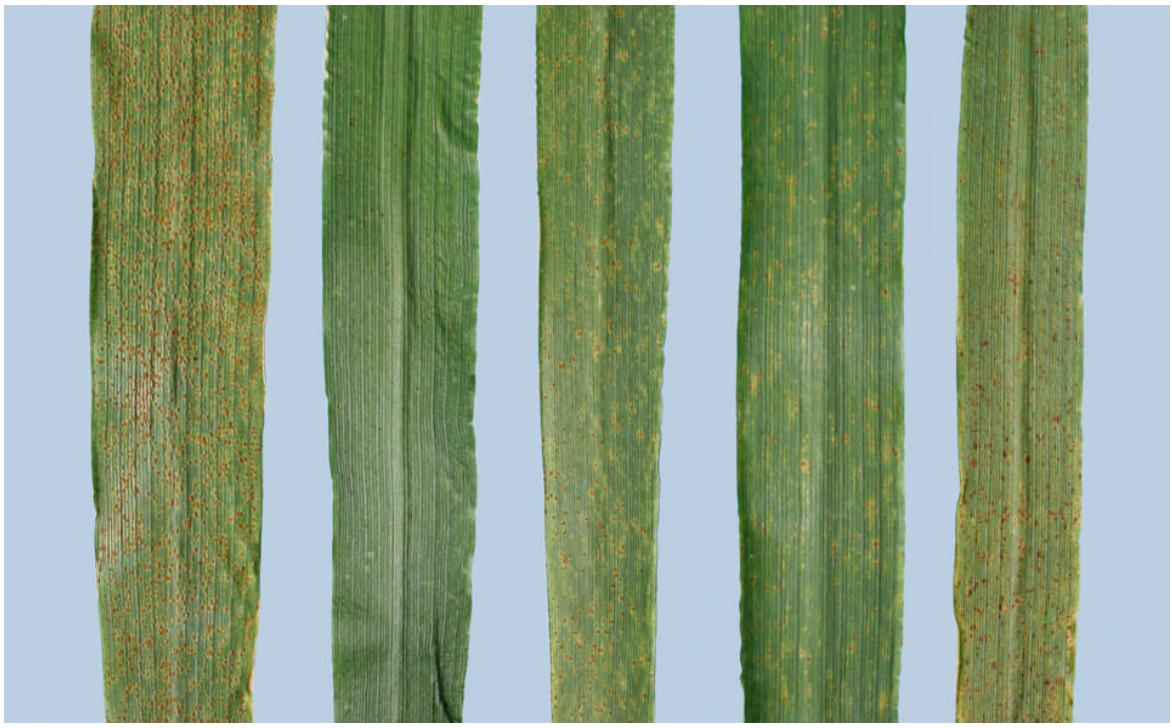


Plate 4.2. Adult plant flag leaf rust responses of (from left) 'Gus' (susceptible male parent), 'Dash', 'Gilbert', 'Athos' and 'Ulandra (NT)' when tested in the field against *Puccinia hordei* pathotype 5653P+.

4.3.1 Inheritance of APR

The BC_1F_2 populations derived from crossing the 10 resistant barley genotypes with the leaf rust susceptible cultivar 'Gus' were assessed for adult plant response to *P. hordei*. The distribution of adult plant responses of BC_1F_2 rows of crosses involving 'Vada', 'Nagrad', 'Gilbert', 'Ulandra (NT)' and 'WI3407' with 'Gus' showed a good fit to a 1:1 (1 segregating for resistance : 1 non segregating susceptible) ratio expected for the segregation of a single locus at both sites (Table 4.3). Despite differences in pathotype and location, the responses of the individual BC_1F_2 row from the cross Vada/2*Gus were similar to those recorded in 2005 (Table 4.3). Disease responses of TR to 20MR were recorded on resistant plants within segregating rows of all populations except those derived from 'Vada', for which ratings of up to 20MS were recorded (data not shown). Each population was also assessed for the inheritance of spike character to ensure that the progeny actually resulted from cross-pollination. The spike character in barley is controlled by V/v or I/i locus, where two-row (VV or II) is dominant over six-row (vv or ii) (Wells, 1962).

Table 4.3. Frequency distribution and Chi-squared analysis of adult plant resistance to *Puccinia hordei* in BC_1F_2 rows derived from crosses of 10 barley genotypes with the susceptible cultivar Gus.

Cross [a]	Karalee					Lansdowne				
	Seg	Non-seg	χ2 1:1	χ2 3:1	No. of seg. loci	Seg	Non-seg	χ2 1:1	χ2 3:1	No. of seg. loci
Vada /2*Gus [b]	90	76	1.18	38.24**	1	-	-	-	-	-
Vada/2*Gus [c]	92	72	2.44	31.25**	1	87	79	0.39	45.18**	1
Athos/2*Gus [c]	59	22	16.90**	0.20	2	42	39	0.11	23.15**	1
Dash/2*Gus [c]	49	17	15.52**	0.02	2	32	34	0.06	24.75**	1
Gilbert/2*Gus [c]	35	25	1.67	8.89**	1	35	25	1.67	8.89**	1
Nagrad/2*Gus [c]	79	71	0.43	39.90**	1	79	71	0.43	39.90**	1
Patty/2*Gus [c]	62	29	11.97**	2.29	2	46	45	0.01	29.02**	1
Pompadour/2*Gus [c]	88	39	18.91**	2.21	2	68	59	0.64	31.18**	1
RAH1995/2*Gus [c]	81	20	36.84**	1.46	2	60	45	2.14	17.86**	1
Ulandra (NT/)2*Gus [c]	47	41	0.41	21.88**	1	41	47	0.41	37.88**	1
WI3407/2*Gus [c]	90	72	2.00	32.67**	1	74	88	1.21	74.28**	1

$\chi 2$ (1 d.f.) at P = 0.01 is 6.64,
[a] Pedigree method according to Purdy *et al*. (1968)
[b] Evaluated in 2005 against *pt*. 5453P- at Karalee
[c] Evaluated in 2006 against *pt*. 5652P+ at Karalee and *pt*. 5653P+ at Lansdowne

All rows scored as segregating for the six row spike character at Karalee also segregated for this trait at Lansdowne, and all rows non-segregating for this trait at Karalee were also non-segregating at Lansdowne, providing a good indication that sowings errors had not occurred in establishing the two trials. The ratio of segregating to non-segregating rows fitted that expected for segregation at a single locus (Table 4.4). The combined data of adult plant responses to *P. hordei* and spike character from individual rows of all populations suggested an independent segregation of these two characters (Appendix 4.1).

The BC_1F_2 rows derived from crosses involving 'Athos', 'Dash', 'Patty', 'Pompadour' and 'RAH1995' with 'Gus' showed monogenic inheritance of APR at Lansdowne, but digenic inheritance at Karalee (Table 4.3). One possible explanation for these results is that these cultivars carry two genes for APR to *P. hordei*, both being effective at Karalee but only one being effective at Lansdowne. To test this hypothesis, a genetic model was constructed to determine the combined phenotypic responses of BC_1F_2 rows grown at the two sites and their ratios (Fig. 4.1). To verify the results predicted in the model, BC_1F_2 rows were classified into four groups based on their adult plant responses to *P. hordei* at the two sites (Table 4.5). Rows segregating for resistance in each population at Lansdowne that displayed a similar response at Karalee, were included in group A. The resistant plants within these segregating rows behaved similarly at both sites with disease responses from TR to 10 MR. Rows included in group B segregated at Karalee and were assessed as non-segregating susceptible at Lansdowne. According to the model, these rows were considered to be heterogeneous for the second locus. The resistant plants within the segregating rows at Karalee gave disease responses of 40 to 50MS. The failure to observe the second resistance locus in these populations at Lansdowne indicated that the disease pressure at this site may have been too high to allow the gene to be scored reliably or that the gene is pathotype specific. The rows included in group C were non-segregating susceptible at both sites and therefore lacked either resistance locus. The distribution of BC_1F_2 rows from all the populations in groups A, B, and C was in the ratio of 2 : 1 : 1 (2 segregating for resistance at both sites : 1 segregation for resistance at only one site : 1 non-segregating susceptible), respectively (Table 4.5), expected for segregation of single locus at both the sites and segregation of an additional independent locus at only one site, as predicted in the genetic model (Fig. 4.1).

Table 4.4. Frequency distribution and Chi-squared analysis of segregation of head character in BC_1F_2 populations derived from crosses of 10 two row barley genotypes with the six row cultivar Gus

Cross [a]	Karalee					Landsdowne				
	Seg	**Non-seg**	**χ2 1:1**	**χ2 3:1**	**No. of seg. loci**	**Seg**	**Non-seg**	**χ2 1:1**	**χ2 3:1**	**No. of seg. loci**
Vada /2*Gus [b]	85	80	0.15	48.54**	1	-	-	-	-	-
Vada/2*Gus [c]	86	78	0.39	44.52**	1	87	78	0.49	43.66**	1
Athos/2*Gus [c]	50	31	4.46*	7.61**	≅1	49	32	3.57	9.09**	1
Dash/2*Gus [c]	31	35	0.24	27.66**	1	37	29	0.97	12.63**	1
Gilbert/2*Gus [c]	34	26	1.07	10.76**	1	32	28	0.27	15.02**	1
Nagrad/2*Gus [c]	84	66	2.16	28.88**	1	85	65	2.67	26.89**	1
Patty/2*Gus [c]	46	45	0.01	29.02**	1	48	43	0.28	24.03**	1
Pompadour/2*Gus [c]	71	56	1.77	24.70**	1	71	56	1.77	24.70**	1
RAH1995/2*Gus [c]	57	54	0.03	11.04**	1	57	54	0.03	11.04**	1
Ulandra (NT/)2*Gus [c]	46	42	0.18	24.24**	1	48	40	0.73	19.64**	1
WI3407/2*Gus [c]	83	79	0.10	48.80**	1	86	76	0.62	41.49**	1

χ2 (1 d.f.) is 3.84 and 6.64, respectively at P = 0.05 and P = 0.01

[a] Pedigree method according to Purdy *et al*. (1968)

[b] Evaluated in 2005 at Karalee

[c] Evaluated in 2006 at Karalee and Lansdowne

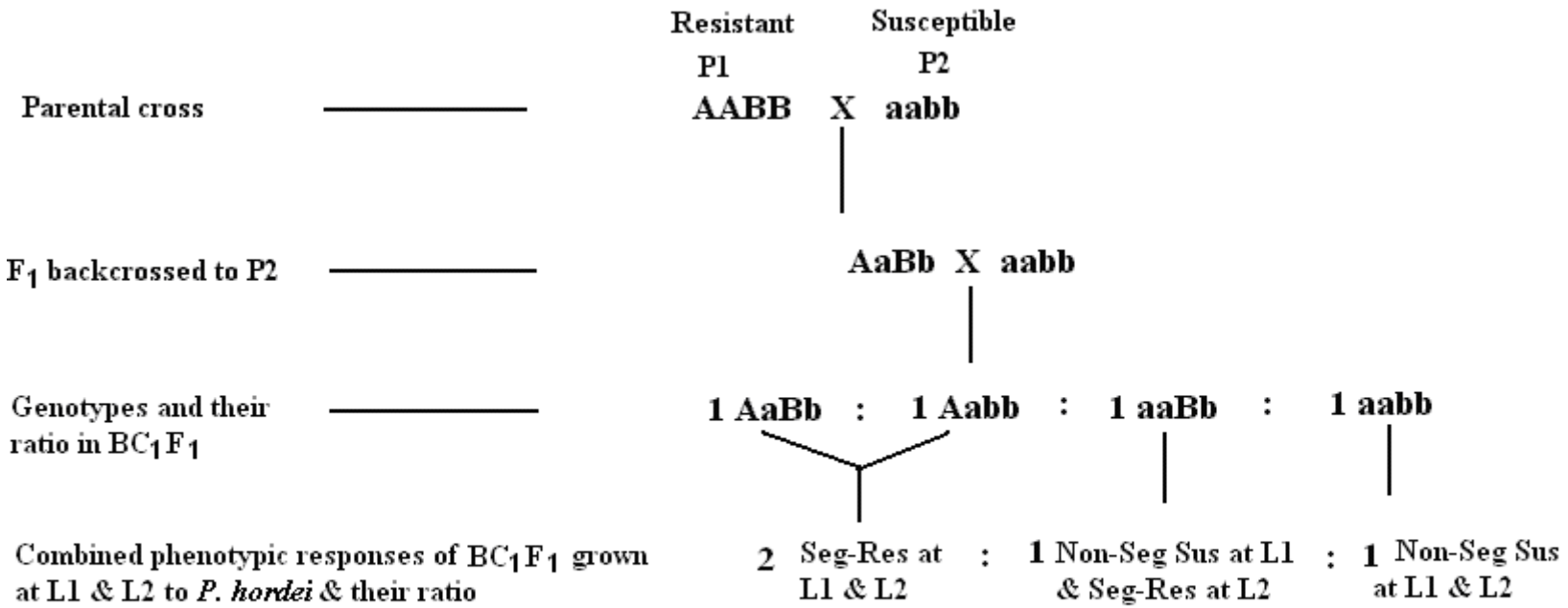


Figure 4.1. A genetic model explaining the phenotypic responses of BC_1F_2 populations grown at two sites to *Puccinia hordei*, assuming locus A is effective at two locations (L1 and L2) while locus B is effective only at location L2

Rows included in group D were segregating at Lansdowne but were scored as non-segregating susceptible at Karalee. Because the single locus that provided the higher levels of resistance at Lansdowne was also effective at Karalee, rows included in this group were assumed to have been misclassified (Table 4.5). They were therefore not included in statistical analyses.

Table 4.5. Distribution of BC_1F_2 rows based on combined adult plant response to *Puccinia hordei* at two field sites

Cross	**Group A** AaBb + Aabb [a]	**Group B** aaBb [a]	**Group C** aabb [a]	**Group D** [b]	**$\chi 2$** [c] **2:1:1**
Athos/2*Gus	40	19	20	2	0.04
Dash/2*Gus	32	17	17	0	0.06
Patty/2*Gus	45	17	28	1	2.69
Pompadour/2*Gus	64	24	35	4	2.17
RAH1995/2*Gus	56	27	28	4	0.03

$\chi 2$ (2 d.f.) at P = 0.05 is 5.99

[a] possible genotype of BC_1F_1 plants based on genetic model (Fig. 4.1)

[b] Not included in Chi-squared analysis

[c] $\chi 2$ based on distribution of BC_1F_2 rows from group A, B and C

Group A – No. of rows scored as segregating for resistance at both the sites

Group B – No. of rows scored as non-segregating susceptible at Lansdowne but segregating for resistance at Karalee

Group C – No. of rows scored as non-segregating susceptible at both the sites

Group D – No. of rows scored as non-segregating susceptible at Karalee but segregating for resistance at Lansdowne

4.3.2 *Allelism tests*

Populations comprising F_2 plants derived from intercrossing the resistant genotypes were evaluated for their adult plant responses to *P. hordei* at the field site Karalee (*pt*. 5652P+). It was at this field site that evidence was obtained for the presence of two genes conferring APR in some genotypes. The F_2 plants were categorised as either resistant or susceptible at anthesis, with disease responses of 60S and higher being regarded as susceptible. The results obtained are summarised in Table 4.6.

With the exception of 'WI3407', no segregation for susceptibility was observed in crosses of 'Vada' with 'Athos', 'Gilbert', 'Nagrad', 'Patty', 'Pompadour' and 'RAH1995', indicating the presence of a common APR gene in these genotypes (Table 4.6 and Appendix 4.2). The ratio of resistant to susceptible plants in the crosses involving 'WI3407' with 'Vada' and 'Nagrad' fitted 15 resistant : 1 susceptible, expected for the segregation of two independent genes (Table 4.6). The distribution of F_2 progeny in 'WI3407'/'Patty' and 'WI3407'/'Pompadour' conformed with the predicted ratio of 63 resistant : 1 susceptible, expected for the segregation of three independent APR genes. The three gene segregation was also expected for RAH1995/WI3407 at this field site. However, the ratio of resistant to susceptible F_2 plants was a good fit to segregation of two independent APR genes. It is likely that plants carrying the gene conferring a low level of APR in this cultivar could have been misclassified as susceptible.

Table 4.6. Leaf rust responses of F_2 populations derived from intercrossing barley cultivars displaying adult plant resistance to *Puccinia hordei*

Cross	Res	Sus	Segregation ratio (Res : Sus)	$\chi 2$
Vada [a]				
X Patty	179	0	No segregation	-
X Pompadour	221	0	No segregation	-
X Nagrad	210	0	No segregation	-
X RAH1995	163	0	No segregation	-
X Athos	171	0	No segregation	-
X Gilbert	127	0	No segregation	-
X WI3407	145	5	15 : 1	2.18
WI3407 [b]				
X Patty	180	6	63 : 1	2.90
X Pompadour	198	7	63 : 1	2.81
X Nagrad	140	13	15 : 1	1.32
X RAH1995	152	14	15 : 1	1.35

$\chi 2$ (1 d.f.) at P = 0.05 is 3.84
'Dash' and 'Ulandra (NT)' were not included in allelism tests
[a] tested in 2005
[b] tested in 2006

4.4 Discussion

The adult plant responses to *P. hordei* of BC_1F_2 rows derived from crossing 'Vada' with the susceptible cultivar 'Gus' at two field sites with different pathotypes in 2006, and at one field site in 2005, confirmed that the APR in 'Vada' was determined by a single locus (Table 4.3). Neervoort and Parlevliet (1978) reported that 'Vada' has partial resistance to *P. hordei*, believed to have been derived from '*H. laevigatum*'. This type of resistance is characterised by a reduced rate of leaf rust development despite a susceptible infection type. The level of partial resistance to *P. hordei* in 'Vada' was assessed using relative latent period, which was governed by the cumulative action of a recessive gene of fairly large effect and four to five genes with small additive effects (Parlevliet, 1976b). Further analyses of partial resistance in 'Vada' using molecular markers located 10 QTLs responsible for the long latent period (Qi *et al.*, 1998, 1999). In a more recent study, Backes *et al.* (2003) located only two QTLs responsible for resistance to *P. hordei* in a population of recombinant inbred lines derived from the cross 'Vada'/'IB 87', when resistance was quantified using Area Under the Disease Progress Curve (AUDPC). The relationship between the monogenic APR identified in the present experiment and the resistance loci identified by Backes *et al.* (2003) and Qi *et al.* (1998, 1999) to *P. hordei* in 'Vada' are not known. The discrepancies in the genetic inheritance of resistance to *P. hordei* in 'Vada' in these three studies may relate to the different scales used to evaluate the resistance, pathotypic differences, and/or environmental differences.

'Vada' carries the powdery mildew resistance gene *MILa*, derived from '*H. laevigatum*' and located on chromosome 2HL (Giese *et al.*, 1993). *MILa* was associated with a single gene prolonging latent period to leaf rust in a DH population developed from the cross 'M1508'/'Sultan' (Jensen and Jørgensen, 1991). *MILa* was also found to be linked to another major gene, *Rdg1a*, conferring resistance to barley stripe disease caused by *Drechslera graminea* (Haar *et al.*, 1989). The mildew resistance provided by *MILa* has been used widely in European barley breeding programmes because it was effective against *E. graminis* f. sp. *hordei* for a number of years (Jørgensen, 1983). However, cultivars derived from '*H. laevigatum*' were generally considered inferior in malting quality, presumably due to the incorporation of the '*laevigatum*' mildew resistance (Swanston, 1987). Based on the results of Haar *et al.* (1989) and Swanston (1987), Jensen and Jørgensen (1991) assumed that *MILa*, *Rdg1a*, gene governing long latent period to leaf rust and genes adversely affecting

malting quality were located close to each other on a small segment of chromosome 2HL, and that this segment was originally transferred from '*H. laevigatum*'. Of the 10 QTLs extending latent period to leaf rust identified by Qi *et al*. (1998) in 'Vada', only one, *Rphq2*, was a major contributor to the partial resistance at seedling as well as adult plant growth stages. This QTL was located close to *MILa* on chromosome 2HL. It is of interest to note that Backes *et al.* (2003) also located a QTL conferring resistance to *P. hordei* on chromosome 2HL, close to *Rdg1a* conferring resistance to leaf stripe disease in 'Vada'. These studies indicate that there might a close relationship between the '*laevigatum*' segment and longer latent period to leaf rust resistance in the cultivar 'Vada'. In the course of the present study, the relationship between *MILa* and the APR gene in 'Vada' was explored by assessing the leaf rust response of cultivar 'Pallas' and the line 'P-23' ('Pallas' isogenic line carrying *MILa*). If the APR gene identified in 'Vada' is very closely associated with *MILa*, 'P-23', developed from the leaf rust susceptible cultivar 'Pallas', could display APR to *P. hordei*. However, the field results suggested that both 'Pallas' and 'P-23' lacked effective resistance against *P. hordei*, indicating a lack of association between *MILa* and the APR identified in Vada (see Chapter 3).

The APR to leaf rust identified in 'Nagrad', 'WI3407', 'Ulandra (NT)' and 'Gilbert' was also found to be conditioned by a single gene. While two genes conferring APR were detected in 'Athos', 'Dash', 'Patty' 'Pompadour' and 'RAH1995' at Karalee, only a single gene was detected at Lansdowne (Table 4.3). It is most probable that one of the APR genes identified in each of these genotypes was difficult to score under higher disease pressure because it conferred very low levels of APR. This effect of this gene was apparently not noticeable at Lansdowne where disease levels were higher (Table 4.2), indicating only one gene providing high levels of APR was operative in these cultivars at all environmental conditions. The tests of allelism showed that the single APR gene detected in Vada was also present in 'Patty', 'Pompadour', 'Athos', 'Nagrad' and 'RAH1995' (Table 4.6). A preliminary mapping study of the APR in 'Pompadour' suggested that the resistance in this cultivar is located on chromosome 5HS, further demonstrating a lack of association between the region of the '*laevigatum*' derived mildew resistance and the gene providing APR to leaf rust (Park, R. F. personal communication). *Rphq4*, a major QTL conferring lower AUDPC and longer latent period to *P. hordei* at adult plant growth stages only in 'Vada' was also located on chromosome 5HS (Qi *et al*.,

1998). Because the location of *Rphq4* was mapped using AUDPC data and it provides resistance only at adult plant growth stages, it would be of interest to know the relationship between this QTL and the APR gene mapped on 5HS in 'Pompadour'. 'Patty', identified as a carrier of APR, was also postulated to carry an uncharacterised seedling resistance gene to *P. hordei* conferring an intermediate infection type (Park *et al.*, 2003). Molecular mapping of seedling leaf rust resistance in a DH population derived from 'Patty' (*Rph*?) and 'Tallon' (*Rph12*) identified two significant loci; one on chromosome 5HL probably corresponding to *Rph12*; and the second of unknown origin on chromosome 5HS. 'Athos', 'Patty', 'Pompadour', and 'Vada' also showed moderately low infection types at seedling growth stages to certain pathotypes of *P. hordei* (Golegaonkar *et al.*, 2006). Therefore, it is possible that the resistance locus from 'Patty' mapped to chromosome 5HS based on seedling phenotypic data is the same as the APR locus in 'Pompadour', and that the locus displays an intermediate infection type at the seedling stage with certain pathotypes under suitable environmental conditions. These results could be similar to the wheat leaf rust APR genes *Lr13* and *Lr34*, which also show intermediate resistance at the seedling stage with certain pathotypes under specific environmental conditions (Dyck *et al.*, 1966; Drijepondt *et al.*, 1991). The APR gene present in 'WI3407' was distinct from those present in 'Vada', 'Patty', 'Pompadour', 'Nagrad' and 'RAH1995' (Table 4.6). These results confirm previous speculation about the uncertain origin of APR in 'WI3407', based on its pedigree information (Golegaonkar *et al.*, 2006).

4.5 Conclusions

The present study described the inheritance of APR to leaf rust in 10 barley genotypes. 'Vada', a cultivar known to have polygenic partial resistance, possessed a single gene conferring APR to *P. hordei* under the field conditions used in this study. Based on allelism studies, it is likely that the APR gene in 'Vada' is also present in 'Patty', 'Pompadour', 'Athos', 'Nagrad', and 'RAH1995', which was found in independent studies to be located on chromosome 5HS. The results obtained in the present study suggested that the APR in 'Vada' may not be related to the partial resistance in this cultivar reported in previous studies. 'Athos', 'Patty', 'Pompadour', and 'Nagrad' were also demonstrated to possess an additional APR gene providing low levels of resistance that was more difficult to phenotype presumably under

situations of high disease pressure. The usefulness of this second gene in breeding barley cultivars resistant to *P. hordei* is presently not known. The APR gene in 'WI3407' differed to that in 'Vada'. It will be worthwhile to conduct mapping studies to find markers closely linked to the APR genes for their efficient use in barley breeding and to determine their relationships with other known resistance genes.

CHAPTER V

Molecular mapping of leaf rust resistance gene *Rph14* in barley

5.1 Introduction

Leaf rust of barley, caused by *Puccinia hordei*, is one of the most destructive diseases in the major barley growing regions of the world (Clifford, 1985). The deployment of monogenic seedling resistance has been practised as an economical and ecologically balanced option to control this disease. Several seedling resistance genes have been identified from cultivated barley and wild barley, of which 19 were designated *Rph1* to *Rph19* (Weerasena *et al.*, 2004). The resistance provided by single *Rph* genes has often been overcome by new pathotypes, believed to have arisen via introduction or mutation (Park, 2003). As a direct consequence, the number of effective *Rph* genes available to breeders is decreasing rapidly, suggesting the need for a new gene deployment strategy (Fetch *et al.*, 1998). In this context, incorporating multiple seedling resistance genes was proposed as a way of increasing the life of the resistance of a cultivar and also to minimise the chance of resistance genes being rendered ineffective (Park, 2003).

Resistance gene *Rph14* was identified in *H. vulgare* accession 'PI 584760' and was shown to be genetically independent of *Rph1* to *Rph13* (Jin *et al.*, 1996) and *Rph15* (Chicaiza *et al.*, 1996). Virulence for *Rph14* has not been detected in Australia (Park, 2003), but has been reported to be rare (3%) in isolates collected from Europe, North America, South America and Africa (Fetch *et al.*, 1998). Deploying this gene in combination with other effective *Rph* genes such as *Rph3*, *Rph7*, *Rph11*, *Rph15* and *Rph18* was therefore proposed as a strategy for long term management of the disease (Park, 2003). However, genotypes carrying multiple genes may show the same phenotypic response to rust as those carrying a single gene due to the masking effect of one gene over another. This can be overcome if pathotypes virulent on individual genes are available. In Australia, virulences for genes *Rph3*, *Rph7*, *Rph11*, *Rph14*, *Rph15* and *Rph18* have not been detected, making the selection of combinations based on these genes difficult (Park, 2003). These drawbacks can be overcome by finding markers closely linked to the genes. In the last two decades, a large number of different marker technologies have been developed, of which, polymerase chain reaction (PCR)-based markers such as simple

sequence repeats (SSR), amplified fragment length polymorphisms (AFLP) and sequence tagged sites (STS) are well suited for marker-assisted-selection (MAS) (Mohler and Singrun, 2004). These markers need very low amounts of DNA for genetic assays and the results produced are highly reproducible in different laboratories. Recently, several mapping studies were conducted on *Rph* genes and DNA markers closely linked to *Rph2* (Borovkova *et al.*, 1997), *Rph5* (Mammadov *et al.*, 2003), *Rph6* (Zhong *et al.*, 2003), *Rph7* (Brunner *et al.*, 2000; Graner *et al.*, 2000), *Rph15* (Weerasena *et al.*, 2004), *Rph16* (Ivandic *et al.*, 1998), *Rph17* (Pickering *et al.*, 1998) and *Rph19* (Park and Karakousis, 2002) were identified. In contrast, the chromosomal location of *Rph14* remains unknown. The present study was therefore conducted to locate *Rph14* to a barley chromosome and to identify PCR based SSR or STS markers that could be used in MAS of this gene in barley breeding programmes.

5.2 Materials and Methods

5.2.1 Plant material

Cultivars 'Baudin' and 'Ricardo' were crossed with the stock possessing *Rph14* ('PI 584760'), and the resulting F_2 and F_3 populations were used to map *Rph14*. The original stock carrying *Rph14* ('PI 584760') (Jin *et al.*, 1996) was kindly provided by B. J. Steffenson (formerly, North Dakota State University, Fargo, ND). For greenhouse tests, seedlings were raised in 9 cm diameter pots containing a soil mix of pine bark fines and coarse sand. Pots were watered with a soluble fertiliser (Aquasol®, Hortico Pty Ltd, Revesby, NSW, Australia) at the rate of 35 g in 3 l of water per 100 pots, prior to sowing. F_2 and F_3 plants were raised by planting 25 to 30 seeds/pot. Seedlings of differential genotypes and parents were raised by sowing clumps (two per pot) of 5 to 7 seeds of each. The pots were transferred to temperature controlled greenhouse chambers (18 ± 2^{o}C) under natural light and maintained until seedlings were ready for inoculation.

5.2.2 Seedling inoculations and disease assessment

Greenhouse inoculations were carried out on 9-day-old seedlings with fully expanded first leaves using urediniospores of *P. hordei* pathotype 5453P- (University of Sydney, Plant Breeding Institute, Cobbitty, Accession 010037 = 560) as described by Park and Karakousis (2002). Inoculated seedlings were incubated for 14-16 h at

ambient temperatures in a misted dark room. The mist was generated by an ultrasonic humidifier. The seedlings were then moved to naturally lit greenhouse chambers at 20 ± 2^{o}C and disease responses were recorded after 10-12 days, using a 0-4 scale infection type (IT) scale (Park and Karakousis, 2002). Infection types of 3 or higher were regarded as indicative of susceptibility.

5.2.3 DNA extraction and bulk preparation

To extract genomic DNA, disease free leaves from 20 to 25 plants of each F_3 line were harvested as a bulk 5 days after disease screening. Genomic DNA was extracted by the CTAB (Cetyltrimethylammonium bromide) method (Doyle and Doyle, 1987) from 20 non-segregating resistant and 20 non-segregating susceptible F_3 lines derived from 'Baudin'/'PI 584760' and the parents. The DNA from all other F_3 lines was extracted using the Mixer Mill Method as outlined by Kota *et al*. (2006) for wheat seed. The concentration of DNA was determined using a spectrophotometer (Nanodrop®, Biolab, Australia), and all samples were adjusted to a final concentration of 50 or 100 ng/µL depending on the requirements of the individual experiment for further molecular analysis. Equal aliquots (5 µL) of DNA from 20 non-segregating resistant and 20 non-segregating susceptible F_3 lines were pooled to produce two DNA bulks for bulk segregant analysis (BSA) (Michelmore *et al.*, 1991). BSA was conducted by Triticarte® (Yarralumla, Australia) using Diversity Array Technology® (DArT) markers as described by Wenzel *et al.* (2007).

5.2.4 SSR and STS analyses

A total of 16 SSR and four STS markers mapped previously to the short arm of barley chromosome 2H were evaluated for polymorphism between the parents as well as between DNA bulks (Table 5.1). Primers generating PCR products polymorphic between the parental lines and bulks were subsequently used to evaluate all F_3 lines of both populations. The relevant details of primers used in the present experiment are given in Table 5.1.

PCR was performed in a volume of 25 µL, containing 1 µL of DNA (~50 ng/µl), 2.5 µL of 10X PCR buffer (Applied Biosystems, Australia), 2.5 µL of 2 mM dNTPs (dATP, dCTP, dGTP, dTTP) (Roche Dignostics, Australia), 1.5 µL of 2.0 mM $MgCl_2$, 1.5 µL (10ng/µL) of forward and reverse primers (Sigma, Australia), 2.5 unit of Taq DNA polymerase (Applied Biosystems, Australia) and 13 µL of ddH_2O.

PCR amplification was performed in a DNA theromocycler (Eppendorf, Germany) programmed for 5 min at 94^{o}C for initial denaturation; followed by 35 cycles each consisting of denaturation at 94^{o}C for 45 s, annealing at 53 to 60 ^{o}C (depending on each primer pair) at 45 s, and extension at 72^{o}C for 45 s; and a final extension at 72^{o}C for 10 min. The annealing temperature for each primer pair was essentially the same as published in the Grain Genes database (http://wheat.pw.usda.gov/GG2/index.shtml), with the exception of 53^{o}C used for Bmag692.

The amplified PCR product (8 µL) and formamide loading buffer (2 µL; 98% formamide, 10 mM EDTA [pH 8.0], 0.05% [wt/vol] Bromophenol blue and 0.05% xylene cyanol) were loaded in 2% agarose gel prepared in 1 X Tris-borate EDTA (TBE) buffer (90 mM Tris-borate + 2 mM EDTA [pH 8.0]) and subjected to electrophoresis at 100 V for approximately 1 h. The separated products were stained with ethidium bromide and visualised under ultra violet light.

The amplified DNA fragments derived from all primer pairs were also separated in 6% polyacrylamide gel following the procedure outlined by Sambrook *et al*. (1989). The gel was pre-run in 1 X TBE buffer for approximately 40 min at 1,600 V, until the gel temperature reached to ~ 50^{o}C. An equal volume of formamide loading buffer (4 µL) was added to each PCR sample (4 µL) and denatured at 94^{o}C for 4 min. The denatured PCR products were chilled on ice and 3 µL of each sample was loaded in each well of polyacrylamide gel. The loaded gel was subjected to electrophoresis at 1500 V for 1.5 to 2 h, depending on the approximate size of the amplified products. The separated DNA fragments were visualised by silver staining.

5.2.5 Chi squared and linkage analyses

The phenotypic data obtained from rust testing the F_2 and F_3 populations were subjected to Chi-squared (χ^2) analysis to confirm the goodness-of-fit of observed ratios to theoretical expectations. The analysis of linkage between *Rph14* and the molecular markers was performed using Map Manager QTXb20 - version 3 (Manly *et al.*, 2001). The Kosambi mapping function was used to convert recombination frequencies to map distances in centi-Morgans (cM).

Table 5.1. Description of 20 DNA markers located on barley chromosome 2HS assessed for potential mapping of *Rph14* in two barley populations.

Marker name	**Marker type**	**Primer sequence (5' – 3')**	**Reference**
GMS003	SSR	TTTCAGCATCACACGAAAGC TTGCATGCATGCATACCC	Struss and Plieske (1998)
EBmac715	SSR	GCGAACATTGTCATGTTAGTA TGTCATGCCAGACCTATG	Ramsey *et al.* (2000)
EBmac607	SSR	GCGAACATTGTCATGTTAGTA AACCTTATGGATTTGGAGG	Ramsey *et al.* (2000)
Bmac518	SSR	ATATGGGTCACACTGAAAATC AGTTTGTTTTTACCAATAAGAGTG	Ramsey *et al.* (2000)
Bmag381	SSR	TTTTATTATTGCATCTAGGGC TATCAAGATCATGACGTCTCA	Ramsey *et al.* (2000)
Bmag341a	SSR	TCATGGAGACCGTTGTAGT CCACAAGCCTCTGTTCTC	Ramsey *et al.* (2000)
Bmac0093	SSR	CGTTTGGGACGTATCAAT GGGAGTCTTGAGCCTACTG	Ramsey *et al.* (2000)
Bmac134	SSR	CCAACTGAGTCGATCTCG CTTCGTTGCTTCTCTACCTT	Ramsey *et al.* (2000)
HVM23	SSR	TCGGTGAAGAAATACGAGGC TCTTTGTAGACCTACCGGTCC	Liu *et al.* (1996)
Bmac132	SSR	AACCTCCATAGTGTAGGGG GTTTGTTCTTTTGATTTTGTTG	Ramsey *et al.* (2000)
Bmac218	SSR	ATTGCATTGATTAACTCCTACA GGGGGAATCTTTGTGTAAG	Ramsey *et al.* (2000)
ABG358	STS	ATTCCAGAACCTCCTCGAC AAGCCACATCAACATAATGC	Kuenzel *et al.* (2000)
Bmag125	SSR	AATTAGCGAGAACAAAATCAC AGATAACGATGCACCACC	Ramsey *et al.* (2000)
MWG2133	STS	CTTTACCACGGTCTATGTCA GGTAAGACATGGAGGACCAT	Kuenzel *et al.* (2000)
ABG459	STS	GCCACCACGCTCTCCATTGT CCACGCTCGCTTGCTGACTC	Rodriguez *et al.* (2006)
HVM36	SSR	TCCAGCCGAACAATTTCTTG AGTACTCCGACACCACGTCC	Liu *et al.* (1996)
Bmag692	SSR	GCAAGGTATCTCTTGTATTTTG TGGCATCTACAATCTAAAACA	Ramsey *et al.* (2000)
GBM1251[a]	SSR	CCAGCAATAACAACGTGTGG TGTCTTTTATTTCCGGAGCG	Varshney *et al.* (2006)
GBM1115[a]	SSR	GTGCCGGTCCTTCATGTC GCCTTCACGTAGTCCCAGAC	Varshney *et al.* (2006)
ABC454	STS	TTCACAGCCGAAACACTTGT GCGTGCGAGGGGAAGGAGAA	Rodriguez *et al.* (2006)

[a] Expressed sequence tag derived SSR

5.3 Results

5.3.1 Genetic analysis

Pathotype 5453P- produced a low IT (;1+CN) on the stock containing *Rph14*, and a susceptible IT (3+) on seedlings of 'Baudin' and 'Ricardo' (Plate 5.1). F_2 seedlings derived from the cross 'Baudin'/'PI 584760', along with the parents and differential genotypes, were tested in the greenhouse against *P. hordei pt.* 5453P-. The infection types of F_2 seedlings were compared with the infection types of parents and those of differential genotypes and each F_2 seedling was categorised as susceptible or resistant. The ratio of resistant to susceptible F_2 individuals was 99 : 53, which showed a significant deviation from a 3 : 1 ratio (χ^2 3 : 1 = 7.90, P < 0.01, 1 df), expected for the segregation of a single gene (Table 5.2). Each susceptible and resistant F_2 plant was marked, transplanted to the field, harvested, and the F_3 progeny were tested against *P. hordei pt.* 5453P-. F_3 lines were scored as either non-segregating resistant, segregating or non-segregating susceptible. The number of F_3 lines included in these three classes conformed to a 1 : 2 : 1 ratio (1 non-segregating resistant : 2 segregating for susceptible : 1 non-segregating susceptible), expected for segregation at a single locus (Table 5.2). Assuming single dominant gene segregation in a population, the progeny of the resistant F_2 plants should have been either non-segregating resistant or segregating and the progeny of susceptible F_2 plants should have been non-segregating susceptible. However, 20 plants that were scored as susceptible in the F_2 were segregating in the F_3, indicating they were heterozygous for *Rph14* and that the F_2 plants had been misclassified. This suggested that the inheritance of *Rph14* tended to be incompletely dominant, which would account for the excess susceptible F_2 plants that resulted in deviation from single gene model. The misclassification of F_2 plants heterozygous for *Rph14*, presumably due to incomplete dominance, was therefore likely the main reason for the deviation from a single gene ratio observed in the F_2 population.

The inheritance of *Rph14* was further confirmed by screening F_3 progeny from the cross 'Ricardo'/'PI 584760' with *P. hordei pt*. 5453P-. The ratio of 22 non-segregating resistant : 47 segregating : 37 non-segregating susceptible observed in F_3 lines conformed to a 1 : 2 : 1 ratio, expected for segregation at a single resistance locus (Table 5.2).

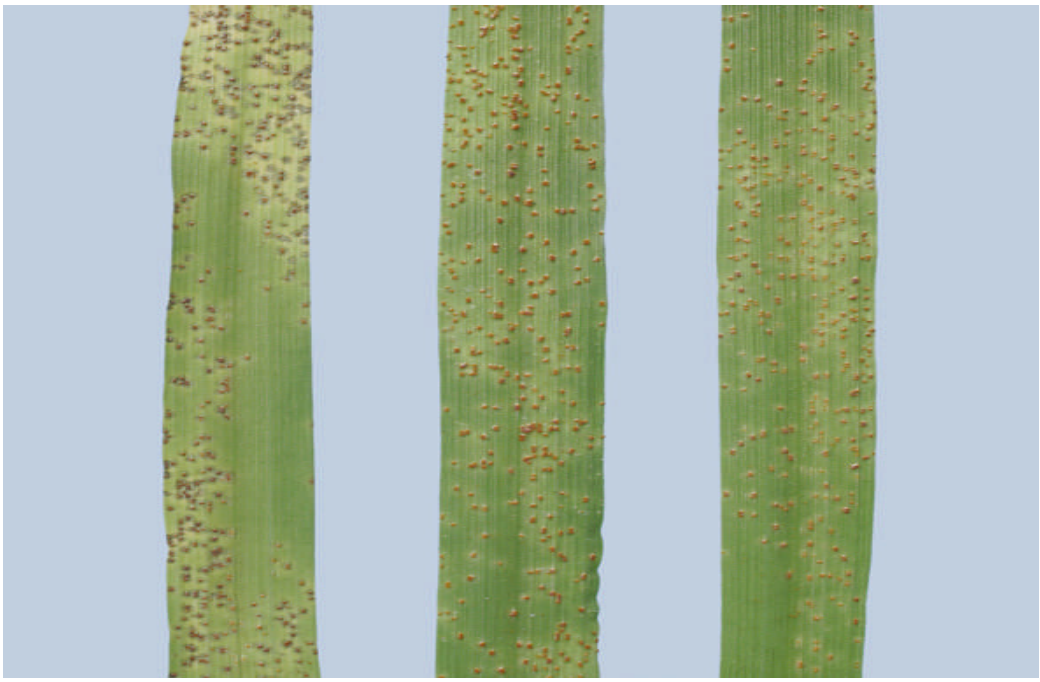


Plate 5.1. Infection types of parents (from left) 'PI 584760 (;1+CN), 'Baudin' and 'Ricardo' (3+) when tested in the greenhouse at seedling growth stages against *P. hordei pt.* 5453P-.

Table 5.2. Observed frequencies of phenotypic classes in F_2 and F_3 populations derived from the crosses 'Baudin'/'PI 584760' and 'Ricardo'/'PI 584760'.

Cross	**Generation**	**Observed frequencies**	**Tested ratio**	**χ2**
		F_2 - R : S [a] **F_3 - NSR : SG : NSS [b]**		
'Baudin'/'PI 584760'	F_2	99 : 53	3 : 1	7.90**
'Baudin'/'PI 584760'	F_3	36 : 81 : 29	1 : 2 : 1	2.43
'Ricardo'/'PI 584760'	F_3	22 : 47 : 37	1 : 2 : 1	5.60

χ^2 (1 d.f.) at P = 0.01 is 6.64 and χ^2 (2 d.f.) at P = 0.05 is 5.99

[a] R = Resistant and S = Susceptible

[b] NSR = Non-Segregating Resistant, SG = Segregating, and NSS = Non-Segregating Susceptible.

The ratio of resistant to susceptible plants within each segregating F_3 line from both the populations was determined and homogeneity χ^2 was calculated before pooling the F_3 lines for molecular analysis. The results suggested that the F_3 lines were segregating for single gene and that the data were homogeneous (Appendix 5.1 and 5.2).

5.3.2 Molecular mapping

Bulk segregant analysis using DArT markers was performed on DNA from the parental genotypes and resistant and susceptible DNA bulks. A total of 2,085 markers were initially screened on parental lines and each marker was scored as either present or absent based on hybridisation intensity. Markers showing contrasting hybridisation intensity between parents were then screened on resistant and susceptible bulks. Amongst 386 informative DArT markers, only eight (bPb-7229, bPb-6755, bPb-2501, bPb-2501, bPb-7906, bPb-3190, bPb-1664, bPb-9925), showed the maximum contrasting hybridisation intensity with DNA from resistant and susceptible bulks (data not presented). The consensus map developed by linking DArT markers with previously mapped SSR, STS and RFLP markers (Wenzel et al. 2006) positioned these markers on short arm of chromosome 2H. The highest contrast was observed with marker bPb-1664, indicating that it had the closest genetic association with *Rph14*. Based on these results, *Rph14* was located on chromosome 2HS (Fig. 5.1).

To identify PCR based markers closely linked to *Rph14*, 16 SSR and four STS markers previously positioned on chromosome 2HS were analysed initially to demonstrate polymorphism between the parents and between the two DNA bulks. Out of the 20 markers, only one SSR marker, Bmag692, generated PCR products that were polymorphic between parents and between DNA bulks. The association between Bmag692 and *Rph14* was determined by screening the marker on 146 and 106 F_3 lines derived from the crosses 'Baudin'/'PI 584760' and 'Ricardo'/'PI 584760', respectively. The segregation of marker Bmag692 on a set of F_3 lines and their parents is shown in Fig. 5.2. Linkage analysis suggested that Bmag692 was linked to *Rph14* with map distances of 2.1 cM in the 'Baudin'/'PI 584760' population and 3.8 cM in the 'Ricardo'/'PI 584760' population.

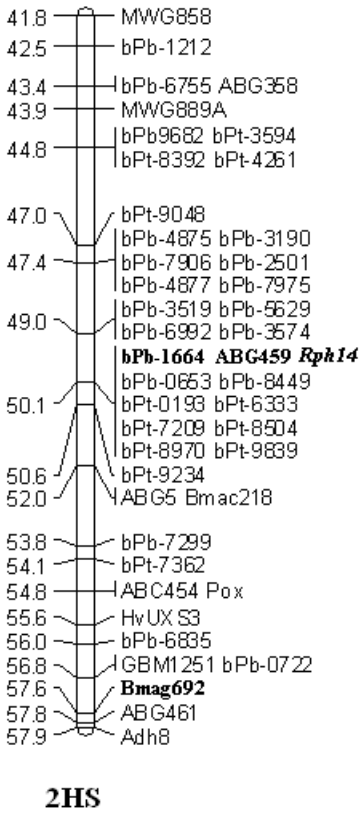


Figure 5.1. Partial consensus map of barley chromosome 2HS developed by linking DArT markers to SSR, RFLP and STS loci (Wenzel *et al*., 2006) and showing the genomic location of *Rph14*. The complete linkage between DArT marker bPb-1664 with *Rph14* was based on the maximum contrasting hybridisation intensity observed between susceptible and resistant DNA bulks with the marker. The SSR marker Bmag692 positioned 57.6 cM from telomere on the consensus map, showed close linkage to *Rph14* when screened on 146 and 106 F_3 lines derived from 'Baudin'/'PI 584760' and 'Ricardo'/'PI 584760', respectively.

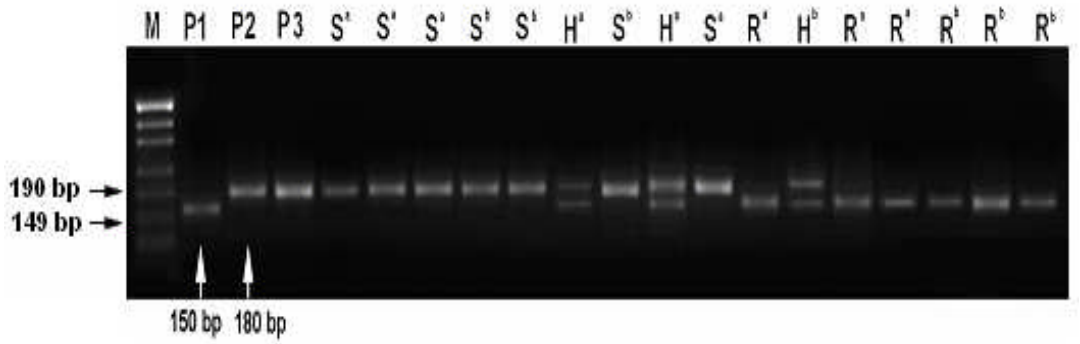


Figure 5.2. Linkage analysis of *Rph14* with marker Bmag692 using F_3 lines from the population 'Baudin'/'PI 584760' (a) and 'Ricardo'/'PI 584760' (b); M - pUC19 / MspI DNA marker, P1 – resistant parent ('PI 584760'), P2 – susceptible parent ('Baudin'), P3 – susceptible parent ('Ricardo'), S – non-segregating susceptible progeny, H – segregating progeny, R – non-segregating resistant progeny. The marker generated PCR products of approximately 150 bp and 180 bp with DNA from resistant and susceptible parents, respectively.

5.4 Discussion

The inheritance of leaf rust resistance in barley accession 'PI 584760', a single resistant plant selection from *H. vulgare* accession 'PI 531901', was previously investigated by Jin *et al.* (1996). The resistance in 'PI 531901' was governed by an incompletely dominant gene that was not allelic to previously designated leaf rust resistance genes *Rph1* to *Rph13*. The new allele symbol *Rph14* was therefore assigned to the single resistance gene identified in 'PI 584760'. In the present study, the inheritance of *Rph14* was confirmed using F_2 and F_3 populations derived from crosses between 'PI 584760' and the cultivars 'Baudin' and 'Ricardo'. Although Baudin carries *Rph12* and Ricardo carries *Rph2* plus an uncharacterised seedling resistance to *P. hordei* (Park, R. F., unpublished), a pathotype virulent on all of these genes was used in the genetic analyses.

The frequency of virulence for *Rph14* in Europe, North America, South America, and Africa was reported by Fetch *et al.* (1998) to be less than 3%. Virulence for this gene has not been detected in Australia (Park, 2003). The widespread effectiveness of *Rph14* means that it could be a useful source of resistance, especially if it is combined with other seedling resistance genes to increase durability. However, selecting plants carrying *Rph14* could be difficult because it is incompletely dominant. Furthermore, a lack of appropriate pathotypes could prevent selecting combinations of *Rph14* with other effective genes using conventional methods (Park, 2003). These problems could be overcome by closely linked PCR-based markers.

In the present study, BSA using DArT markers positioned *Rph14* on chromosome 2HS. Previous mapping studies of leaf rust resistance genes in barley have shown that *Rph15* (Weerasena *et al.*, 2004), *Rph16* (Ivandic *et al.*, 1998) and *Rph17* (Pickering *et al.*, 1998) are also located on chromosome 2HS. These genes are also potentially important in barley breeding in Australia because they confer resistance to all known Australian pathotypes of *P. hordei* (Park, R.F. Personal communication). Chicaiza *et al.* (1996) demonstrated independent segregation of *Rph14* in a cross between *H. spontaneum* accessions 'PI 355447' (carrying *Rph15*) and 'PI 584760'. However, the relationships of *Rph14* with the genes *Rph16* and *Rh17*, also located on chromosome 2HS, have not been investigated. Weerasena *et al.* (2004) studied the genetic relationship between *Rph15* and the gene *Rph16* and

demonstrated that they are allelic. Therefore, *Rph16* should not be associated with *Rph14* because its allele (*Rph15*) was shown to be genetically independent from *Rph14*. The gene *Rph17* was characterised in a recombinant line derived from a cross between *H. vulgare* and *H. bulbosum*. This recombinant line also carries the powdery mildew resistance gene *Mlhb*, which is tightly linked to *Rph17* (Pickering *et al.*, 1998). Therefore, knowledge of any potential linkage between *Rph17* and *Rph14* could be useful in barley breeding to select combinations of *Rph14*, *Rph17* and *Mlhb*.

BSA is the most efficient method to identify markers associated with a target locus, because it overcomes the need to construct a complete genetic map. BSA was used successfully in several previous studies to locate resistance genes in barley using PCR based markers (Poulsen *et al*., 1995; Mammadov *et al.*, 2003; Weerasena *et al*., 2003; Zhong *et al*., 2003; Agrama *et al*., 2004; Genger *et al*., 2005). Recently, the suitability of hybridisation based DArT markers for BSA in barley was discussed by Wenzel *et al.* (2007). BSA using PCR based markers such as SSR, STS, RFLP and AFLP analyses the polymorphism between parents and bulks, thereby reducing the number of marker assays required to identify the location of the target locus. In contrast to gel-based markers, BSA using DArT has the advantage of identifying the linkage relationships of individual markers with the trait of interest without having to screen all individuals from the population. Because the bulks comprise the highest contrasting frequency of the two allelic states of the target locus, markers associated with the locus will also show the maximum contrasting hybridisation intensities. This information is useful in reducing the number of markers required for precise mapping of the target locus and in identifying markers linked to it.

To identify the efficiency and precision of BSA-DArT method in locating major genes in barley, Wenzel *et al.* (2007) compared two DNA bulks from ‘Steptoe’/‘Morex’ DH lines that segregated for gene *mPub*, controlling pubescent leaves, and bulks from ‘Dayton’/‘Zhepi’ DH lines that segregated for the aluminium tolerance gene *Al*. Genes *mPub* and *Al* were previously mapped on chromosomes 3H and 4H, respectively, using all individuals of each population. BSA-DArT data revealed that these genes were positioned on the same chromosomes within a short distance (~5 cM) from previously mapped locations (Wenzel *et al.*, 2007). In the present study, SSR marker Bmag692 was closely linked to *Rph14*. The consensus map developed using BSA data located this marker at a map distance of 7.5 cM from the DArT marker bPb-1664, which was completely associated with *Rph14*. This

result further demonstrates the efficiency of the method in identifying the precise genomic location of the target locus. The key disadvantage of the method is that it requires costly laboratory set ups and specialised equipment for regular genetic assays. However, given that the barley DArT markers were recently sequenced (Wenzel, P. Personnel communication), the sequence information of DArT markers closely linked to the target locus can now be used to develop PCR based STS markers for regular genetic assays.

5.5 Conclusion

Information on the genomic location of the potentially useful *Rph* genes and molecular markers closely linked to these genes are useful in breeding for leaf rust resistance. This information can be used to select genes that are difficult to phenotype, or to combine two or more *Rph* genes in single genotype. In the present study, BSA using DArT markers positioned gene *Rph14* on chromosome 2HS. Further analyses with SSR and STS markers corresponding to chromosome 2HS demonstrated that the SSR marker Bmag692 was closely associated with *Rph14*. The close linkage and co-dominance of Bmag692 mean that it will be useful in selection for *Rph14*. The efficiency of using this marker in MAS could be improved by either identifying a second marker flanking *Rph14* or by further fine mapping studies for cloning the gene.

CHAPTER VI

Genetic and molecular analyses of resistance to a form of *Puccinia striiformis* in barley

6.1 Introduction

Stripe rust, caused by *P. striiformis*, is an economically important disease of wheat and barley, causing significant yield losses under epidemic conditions in susceptible cultivars (Line, 2002). The pathogens causing stripe rust in barley (*P. striiformis* f. sp. *hordei*; *Psh*) and wheat (*P. striiformis* f. sp. *tritici*; *Pst*) are regarded as separate *formae speciales* although the host ranges of both overlap (Stubbs, 1985). In Australia, *Pst* was first detected in 1979 and it continues to be a serious disease of wheat (Wellings, 2007). *Pst* is not an economically important disease of barley in this region because most Australian barley cultivars are resistant to locally occurring isolates of *P. striiformis* (Wellings, 2007). *Psh* is not present in Australasia, and a majority of barley cultivars from this region were susceptible to race 24 of *Psh* when field screened at CIMMYT, Mexico (Wellings *et al*., 2000b).

Wellings *et al.* (2000a) reported a new variant of *P. striiformis* causing stripe rust on some barley genotypes and wild *Hordeum* species in Australia. They considered it to be different from *Pst* and *Psh*, and temporarily designated it Barley Grass Stripe Rust (*BGYR*). This pathogen was partially virulent on the wheat stripe rust differential 'Chinese 166' (*Yr1*) and was pathogenic to a small group of barley cultivars, most notably 'Skiff' and some derivative genotypes (Wellings *et al.*, 2000a). Molecular studies conducted on Australian isolates of *Pst* and *BGYR* concluded that *BGYR* was genetically distinct from *Pst* and other *P. striiformis* taxa, suggesting a new introduction into Australia (Keiper *et al.*, 2003). Following its first detection in 1998, *BGYR* has been frequently detected on wild *Hordeum* spp. and rarely from commercial barley crops in New South Wales (NSW) and Victoria in Australia (Wellings *et al.*, 2000a).

The present study was conducted to determine the responses of Australian barley cultivars to *BGYR* and to study the inheritance and genomic location of resistance in selected barley cultivars. These studies should provide a basis to

determine the current and potential future impact of this pathogen on the barley industry in Australia.

6.2 Materials and methods

6.2.1 Genetic material

The pedigrees and years of release of 60 Australian and two exotic barley cultivars used in the present experiments are given in Table 6.1. Three doubled haploid (DH) populations developed from the crosses 'Clipper'/'Sahara 3771', 'Franklin'/'Skiff', and 'Tantangara'/'Tilga', and five F_2 and F_3 populations derived from the crosses 'Baudin'/'Sudan', 'Tantangara'/'Sudan', 'Stirling'/'Sudan', 'Ketch'/'Sahara 3771' and 'Skiff'/'Sudan' were used to study inheritance of resistance to *BGYR*. F_2 populations developed from the crosses 'Tantangara'/'Skiff', 'Tantangara'/'Sahara' and 'Skiff'/'Sahara' were used in tests of allelism.

Table 6.1. Pedigrees and years of release of 60 Australian and two exotic barley cultivars assessed for response to Barley Grass Stripe Rust.

Cultivar [a]	**Year**	**Pedigree**
Arapiles	1993	Noyep/Proctor//CI3576/Union/4/Kenia/3/Research/2/Noyep/ Proctor/5/Domen
Bandulla	1981	Prior/Lenta//Noyep/Lenta
Barque	1997	Triumph/Galleon
Baudin	2002	Stirling/Franklin
Binalong	2001	Blenheim//Skiff/O'Conner
Brindabella	1993	Weeah/CI7115//HCB27/3/Jadar II/4/Cantala
Bussell	1967	Prior/Ymer
Cantala	1981	Kenia/Erectoides 16
Clipper	1968	Proctor/Prior A
Cowabbie	2002	(AB6/Franklin//Franklin-early)/3/(Rubin/Skiff-early)
Cutter	1979	Proctor/Prior A
Dash	1995	Chad/Joline//Cask
Dhow	2002	WI2808//Skiff/Haruna Nijo 9
Dictator	1997	Reselection of USDA accession CI2204
Fitzgerald	1997	Onslow/Tas 85-466
Franklin	1989	Shannon/Triumph
Gairdner	1997	Onslow/Tas 83-587
Galaxy	1993	24719DB/Robin SIB
Galleon	1981	Clipper/Hiproly//3*Proctor/CI3576
Gilbert	1992	Reselection of Koru
Grimmett	1982	Bussel/Zephyr
Hamelin	2002	Stirling/Harrington

Harrington	1981	Klages/3/Gazelle/Betzes//Centennial
Kaputar	1993	5604/1025/3/Emir/Shabet//CM67/4/F3 Bulk Hip
Keel	2000	CPI18197/WI12645
Ketch	1969	Noyep/Lenta
Lara	1971	Research/Lenta
Lindwall	1997	Triumph/Grimmett
Mackay	2002	Cameo/Koru
Malebo	1981	Selection from CPI11083 (Palladium WWB 18)
Maritime	2004	(details unavailable)
Milby	2002	(AB6/Franklin//Franklin-early)/3/(Rubin/Skiff-early)
Molloy	1996	Golden Promise/WI2395(WAR12-38)/4/(72S:267)XBVT210/3/(66S08-4)Atlas57//(A14)Prior/Ymer(82S837)/O'Connor
Moondyne	1987	Dampier//(A14)Prior/Ymer/3/Kristina/(70S20-20)/4/(73S13)Clipper/Tenn-65-117
Mundah	1995	O'Connor/Yagan
Namoi	1993	Sultan/Nackta//RM1508/Godiva
Noyep	1959	Single plant selection from Prior's Chevalier
O'Conner	1984	Proctor/CI3576(WI2231)/3/(XBVT212)Atlas 57//(A14)Prior/Ymer
Onslow	1989	Forrest/Aapo
Parwan	1978	Plumage Archer/Prior//Lenta/3/Research/Lenta
Picola	1998	75031/Elgina(75031=Noyep/Prior//CI3576/Union/Kenia/4/Research/Noyep/Prior
Prior	1905	Selected from Chevalier
Sahara 3771 [a]	1925	Algerian land cultivar
Schooner	1983	Proctor/Prior A//Proctor/CI3576
Shannon	1979	Proctor*4/Ethiopian line CI3208-1
Skiff	1988	Abed Deba/3/Proctor/CI-3576//CPI-18197/Beka/4/Clipper/Diamant/Proctor/CI-3576
Sloop	1997	RL1577/84/Schooner
Stirling	1981	Dampier/Prior/Ymer/3/Piroline
Sudan [a]	unknown	Unknown
Tallon	1991	Triumph/Grimmett
Tantangara	1996	AB6/Skiff (AB6 is *H. spontaneum* CPI71283/4*Clipper)
Tilga	1997	Forrest/Cantala
Torrens	2002	Galleon/CIMMYT 42002
Tulla	2002	Skiff/FM437
Ulandra	1987	Warboys/Alpha
Weeah	1968	Prior/Research
Windich	1989	Atlas 57//(A16)Prior/Ymer(68S17-75)/3(B6729)Prior/Lenta//Noyep/Lenta
Wyalong	1998	Schooner/Stirling
Yagan	1989	Unknown
Yambla	1998	Skiff/FM437
Yerong	1990	M22/Malebo

[a] Exotic barley cultivars

6.2.2 Inoculation procedures

For greenhouse tests, seedlings of all populations and parental lines were raised in 9 cm diameter pots containing a mixture of pine bark fines and coarse sand. The pots were watered prior to sowing with a soluble fertiliser (Aquasol®, Hortico Pty. Ltd., Revesby, NSW, Australia) at the rate of 35 g per 3 L for 100 pots. Approximately 25 to 30 seeds of each F_2 plant or each F_3 line were sown per pot. Lines of DH populations and parents were sown in clumps (two per pot) of 5 to 7 seeds each. Seedlings with a fully expanded first leaf (about 8 to 10-day-old) were inoculated with *BGYR* isolate 981549. This isolate was collected from infected barley grass in Victoria during the annual cereal rust pathogenicity survey conducted by Plant Breeding Institute, Cobbitty (PBIC), University of Sydney, in 1998 (C. R. Wellings, Pers. Comm.). The inoculated seedlings were covered with plastic hoods and incubated overnight at 11 ± 2°C. The seedlings were then transferred to naturally lit greenhouse rooms where the temperature was maintained at 17 ± 2°C.

Field tests were conducted at the field site Breakwell, near the Plant Breeding Insititute, during the cropping seasons of 2006 and 2007. Approximately 10 to 15 seeds of each line of the 'Clipper'/'Sahara 3771' DH population were sown in 1 M rows. Lines of the moderately susceptible cultivar 'Maritime' were also sown after every fifth line and surrounding the experimental area for inoculum increase. Field inoculations were carried out by misting spreader rows with fresh urediniospores of *BGYR* isolate 981549 suspended in Shellsol® oil (10mg/200ml) using an ultra-low-volume applicator (Microfit®, Micron Sprayer Ltd., Bromyard, Herefordshire, UK). Four successive inoculations were carried out on afternoons when there was a possibility of overnight dew. Random sites of 15 to 20 inoculated plants of spreader rows were sprinkled with water and covered overnight with plastic hoods to ensure infection in cases where dew formation was inadequate. The experimental plots were irrigated regularly every week when there was no natural rainfall.

6.2.3 Disease assessment

Disease assessment of seedlings was carried out at 16 to 18 days post inoculation, using a 0-4 infection type (IT) scale as described for *Pst* by McIntosh *et al.* (1995). Infection types of 3 or higher were considered to indicate compatibility. The adult plant responses to *BGYR* were assessed post-anthesis using a 0-9 scale as

described by Wellings *et al*. (2004) for *Pst*, where disease rating scores 7 and above were categorised as susceptible.

6.2.4 Statistical and molecular analyses

The data obtained from the rust tested F_2, F_3 and DH populations were subjected to Chi-squared ($\chi 2$) analysis to confirm the goodness-of-fit of observed ratios to theoretical expectations. A genetic linkage map, produced for the 'Clipper'/'Sahara 3771' DH population using RFLP and SSR markers (Karakousis *et al.*, 2003b), was used in analysing the phenotypic data for this population. A computer program, Map Manager QTXb20 - version 3 (Manly *et al.*, 2001) was used to perform interval and linkage mapping. The Kosambi mapping function was used to convert recombination frequencies to map distances in centi-Morgans (cM). In order to determine the significant level of QTL effects, the dataset was analysed by setting 2000 permutations at 5 cM steps. The significant associations between major QTL governing rust resistance and marker loci were also determined by likelihood ratio statistics (LRS). The logarithm of odd ratio (LOD) was calculated by dividing the LRS value by 4.61 (Lander and Botstein, 1989).

6.3 Results

The seedling responses of the barley cultivars to *BGYR* are presented in Table 6.2. The cultivars were classified broadly into resistant or susceptible based on their infection type (IT) response. Among the 62 cultivars tested, only nine ('Clipper', 'Cutter', 'Keel', 'Ketch', 'Maritime', 'Prior', 'Skiff', 'Tantangara' and 'Sudan') showed susceptibility and were therefore concluded to lack effective seedling resistance against the *BGYR* isolate used. Seedling responses were used to design and select genetic populations to determine the inheritance of resistance to *BGYR* in certain barley cultivars. Given the seedling susceptibility of 'Clipper', the resistance of 'Sahara 3771', and the existence of a DH population for which a molecular map was available, the inheritance of resistance of 'Sahara 3771' was investigated at seedling and adult plant growth stages.

Table 6.2. Infection types of 60 Australian and two exotic barley cultivars when inoculated with Barley Grass Stripe Rust isolate 981549.

Cultivar	**Infection type** [a]	**Cultivar**	**Infection type** [a]
Arapiles	;1-CN	Molloy	;;1=C
Bandulla	0;N	Maritime	3C
Barque	0;N	Milby	;1-C
Baudin	0;N	Moondyne	00;N
Binalong	00;C	Mundah	00;
Brindabella	;;N	Namoi	1=C
Bussell	0	Noyep	;;N
Cantala	0;CN	O'Conner	00;N
Cask	2C	Onslow	00;N
Clipper	33+	Parwan	;1=NC
Cowabbie	;1=C	Picola	;NC
Cutter	3C	Prior	3C
Dash	0;N	Sahara 3771	1++2CN
Dhow	;2C	Schooner	;;N
Dictator	2C	Shannon	;N
Fitzgerald	0;N	Skiff	3C
Franklin	;1=C	Sloop	00;N
Gairdner	00;	Stirling	0;N
Galaxy	0;CN	Sudan	3+
Galleon	1-C	Tallon	00;
Gilbert	;1=CN	Tantangara	3C
Grimmett	0;N	Tilga	0;N
Hamelin	0;N	Torrens	1C
Harrington	;1=NC	Tulla	0;N
Kaputar	;1-C	Ulandra	00;NC
Keel	3C	Weeah	;;N
Ketch	33+	Windich	;1-CN
Lara	0;N	Wyalong	;N
Lindwall	0;N	Yagan	;1- C
Mackay	0;N	Yambla	;0NC
Malebo	1=C	Yerong	0;

[a] 0-4 scale as described for *Pst* by McIntosh *et al.* (1995).

6.3.1 Genetic analysis

Three DH populations derived from crossing the resistant cultivars 'Franklin', 'Tilga' and 'Sahara 3771' with the susceptible cultivars 'Skiff', 'Tantangara' and 'Clipper', respectively, were assessed for response to *BGYR* at seedling growth stages. Infection type responses of the parental cultivars are illustrated in Plate 6.1

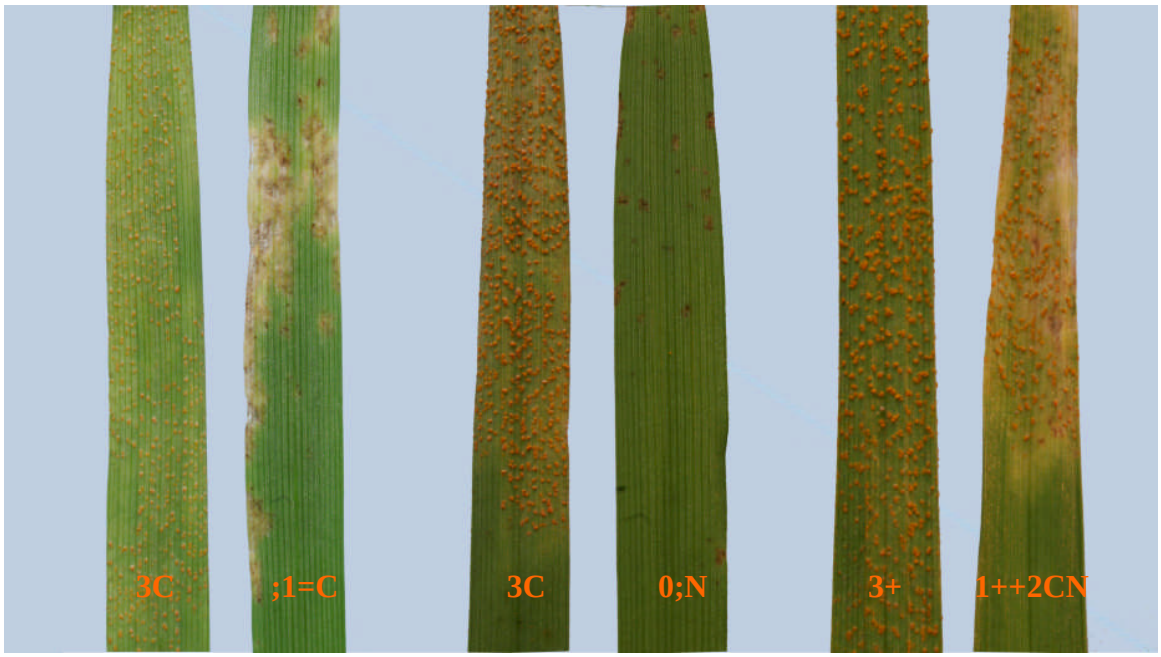


Plate No. 6.1. Infection types of parental cultivars of DH populations (from left) 'Skiff', 'Franklin', 'Tantangara', 'Tilga', 'Clipper'and 'Sahara 3771' when tested at seedling growth stages against Barley Grass Stripe Rust isolate 981549 in the greenhouse.

Certain lines showed ITs higher than 'Skiff' and 'Tantangara' in the 'Franklin'/'Skiff' and 'Tantangara'/'Tilga' DH populations, respectively. These lines were classed as susceptible. Lines showing all other ITs, including those similar to 'Skiff' and 'Tantangara', were scored as resistant. The segregation pattern in these populations indicated that 'Skiff' and 'Tantangara' also possess resistance to *BGYR*. The frequencies of resistant to susceptible DH lines observed in 'Franklin'/'Skiff' fitted a 7 : 1 ratio, expected for the segregation of three resistance genes (Table 6.3). The 'Tantangara'/'Tilga' DH population was scored as 119 resistant : 30 susceptible, which supported segregation of two resistance genes (Table 6.3). The DH population derived from the cross between 'Clipper' and the resistant cultivar 'Sahara 3771' segregated 65 resistant : 85 susceptible, which was a good fit for segregation of a single gene providing resistance to *BGYR* (Table 6.3). The single resistance gene from Sahara 3771 conferred IT of 1++2CN (Plate 6.3).

F_2 and F_3 populations developed from the crosses 'Skiff'/'Sudan', 'Tantangara'/'Sudan' 'Baudin'/'Sudan', 'Stirling'/'Sudan' and 'Sahara 3771'/'Ketch' were also assessed for responses to *BGYR* at seedling growth stages. The parental ITs are presented in Plate 6.2.

Table 6.3. Frequencies of resistant and susceptible lines of three DH populations of barley when inoculated with Barley Grass Stripe Rust isolate 981549.

DH Population	Observed frequency		Tested ratio (Res : Sus)	$\chi 2$	P [a]
	Res	Sus			
Clipper/Sahara 3771	65	85	1 : 1	2.67	0.10
Franklin/Skiff	136	25	7 : 1	1.35	0.25
Tantangara/Tilga	119	30	3 : 1	1.88	0.17

$\chi 2$ (1 d.f.) at P = 0.05 is 3.84, [a] P = Probability

Individual F_2 plants from these populations were scored as either resistant or susceptible. The resistant and susceptible F_2 plants in all populations except 'Sahara 3771'/'Ketch' conformed to a 3 resistant : 1 susceptible ratio, indicating segregation of a single dominant resistance gene (Table 6.4). The distribution of 39 resistant : 108 susceptible in F_2 plants from the cross 'Sahara 3771'/'Ketch' however conformed to 1 resistant : 3 susceptible ratio, indicating segregation of a single recessive gene (Table 6.4).

Table 6.4. Frequencies of resistant and susceptible F_2 seedlings derived from crosses involving four Australian and an Algerian barley cultivars against Barley Grass Stripe Rust isolate 981549 in seedling tests.

Cross	Observed frequency		Tested ratio (Res : Sus)	χ^2	P [a]
	Res	Sus			
Baudin/Sudan	63	16	3 : 1	0.95	0.33
Sahara 3771/Ketch	39	108	1 : 3	0.18	0.67
Stirling/Sudan	148	35	3 : 1	3.37	0.07
Skiff/Sudan	131	42	3 : 1	0.05	0.82
Tantangara/Sudan	120	37	3 : 1	0.17	0.68

$\chi 2$ (1 d.f.) at P = 0.05 is 3.84, [a] P = Probability

In order to determine genotypes of the F_2 plants classified resistant and susceptible, F_3 progeny obtained from individually harvested F_2 plants were inoculated with *BGYR* isolate 981549 and scored as non-segregating resistant, segregating or non-segregating susceptible. The ratio of F_3 lines derived from the crosses 'Sahara 3771'/'Sudan', 'Skiff'/'Sudan' and 'Tantangara'/'Sudan' conformed to a ratio of 1 non-segregating resistant : 2 segregating : 1 non-segregating susceptible ratio, confirming the F_2 prediction of a single resistance gene segregation in each cross (Table 6.5).

Plate No. 6.2. Infection types of parental cultivars (from left) ‘Sudan’, ‘Skiff, ‘Tantangara’, ‘Baudin’ and ‘Stirling’ when tested in greenhouse at seedling growth stages against Barley Grass Stripe Rust isolate 981549.

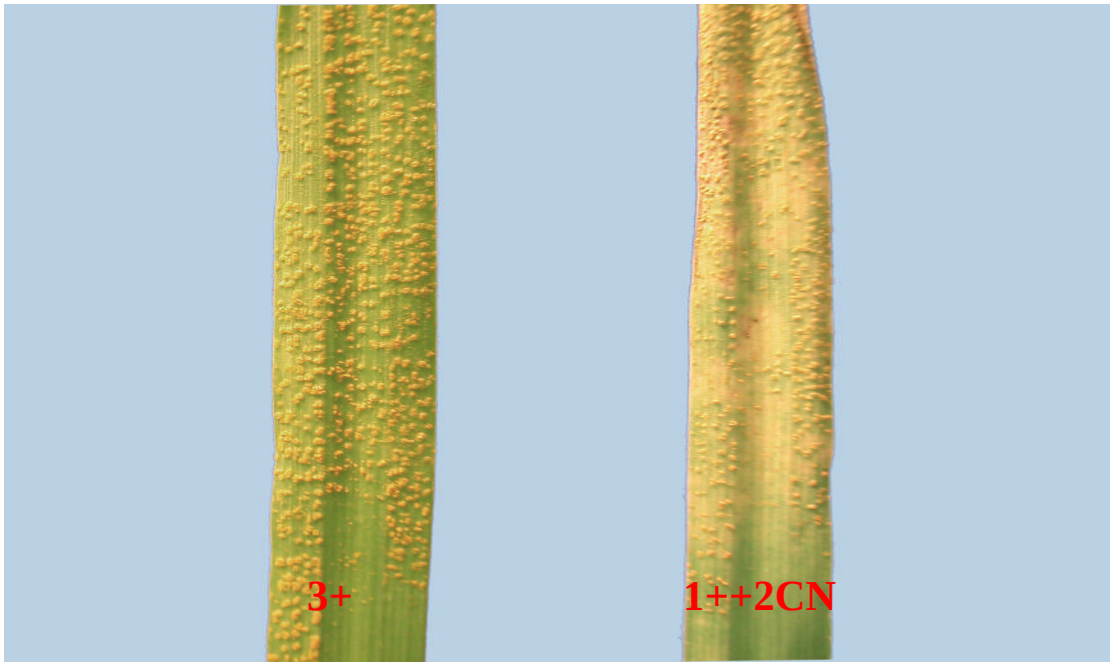


Plate No. 6.3. Infection types of (from left) ‘Ketch’ and ‘Sahara 3771’ when tested in the greenhouse at seedling growth stages against Barley Grass Stripe Rust isolate 981549.

Similar results based on F_2 observations were expected for the F_3 lines derived from the crosses 'Baudin'/'Sudan' and 'Stirling'/'Sudan'. However, the F_3 lines from these populations conformed to a ratio of 7 non-segregating resistant : 8 segregating : 1 susceptible, expected for segregation of two independent genes (Table 6.5).

Table 6.5. Frequencies of resistant and susceptible F_3 seedlings derived from crosses involving four Australian and an Algerian barley cultivars against Barley Grass Stripe Rust isolate 981549 in seedling tests.

Cross	**Observed frequency**			**Tested ratio**	χ^2	**P** [a]
	Res	**Seg**	**Sus**	**(Res : Seg : Sus)**		
Baudin/Sudan	32	29	2	7 : 8 : 1	1.87	0.39
Sahara 3771/Ketch	25	46	36	1 : 2 : 1	4.36	0.11
Stirling/Sudan	49	82	11	7 : 8 : 1	4.99	0.08
Skiff/Sudan	32	70	21	1 : 2 : 1	4.31	0.12
Tantangara/Sudan	39	59	25	1 : 2 : 1	3.39	0.18

$\chi 2$ (2 d.f.) at P = 0.05 is 5.99, [a] P = Probability

The F_3 progeny of several F_2 plants scored as susceptible were segregating, indicating that the F_2s had been misclassified. The resistant plants within these segregating lines conferred a low level of resistance (IT 2+C), which could have contributed to misclassification of F_2 plants carrying this gene.

6.3.2 Tests of allelism

F_2 populations derived from intercrossing three resistant cultivars were assessed in the greenhouse for response to *BGYR* isolate 981549 at seedling growth stages. No susceptible segregates were observed in the cross 'Skiff'/'Tantangara', indicating the presence of a common seedling resistance gene in these cultivars (Table 6.6). The distribution of resistant to susceptible F_2 plants derived from the crosses 'Sahara 3771'/'Skiff' and 'Sahara 3771'/'Tantangara' conformed to 15 resistant : 1 susceptible ratio, indicating segregation of two independent genes (Table 6.6).

Table 6.6. Distribution of seedlings resistant and susceptible to *BGYR* in F_2 populations derived from the crosses 'Skiff'/'Tantangara', 'Sahara 3771'/'Skiff' and 'Sahara 3771'/'Tantangara'.

Cross	Observed Frequency		Tested ratio Res : Sus	χ^2	P [a]
	Res	Sus			
Skiff/Tantangara	131	0	No segregation	-	-
Sahara 3771/Skiff	91	11	15 : 1	3.58	0.06
Sahara 3771/Tantangara	109	12	15 : 1	2.78	0.10

$\chi 2$ (1 d.f.) at P = 0.05 is 3.84, [a] P = Probability

6.3.3 Mapping seedling resistance in Sahara 3771

A single seedling resistance gene identified in 'Clipper'/'Sahara 3771' DH population (Table 6.3) confirmed as being recessive in the genetic study of F_2 plants from the cross 'Sahara 3771'/'Ketch', was mapped by integrating phenotypic data with available molecular marker data provided by Karakousis *et al*. (2003b). The trait data obtained from individual DH lines was compared with the molecular data, and the frequency of parental and recombinant classes with individual marker loci was computed to establish linkage. The linkage between marker loci and the resistance gene and within marker loci was estimated by converting recombination frequency into genetic distance using the Kosambi mapping function. Linkage analyses between the DNA markers and phenotypic responses to rust suggested that the seedling resistance gene was located on the long arm of chromosome 1 (7H) and was flanked by RFLP markers wg420 and cdo347 at map distances of 12.8 cM and 21.9 cM, respectively (Fig. 6.1). The gene was tentatively designated *Bgyr1*.

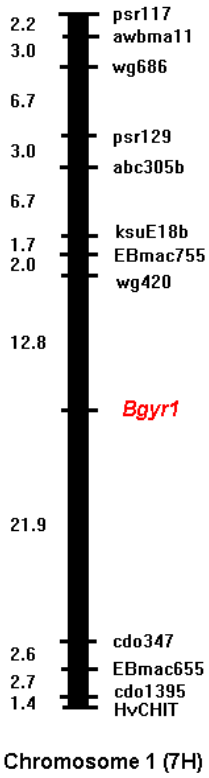


Figure 6.1. Partial map of barley chromosome 1 (7H) showing the genomic location of seedling gene *Bgyr1* providing resistance to Barley Grass Stripe Rust isolate 981549.

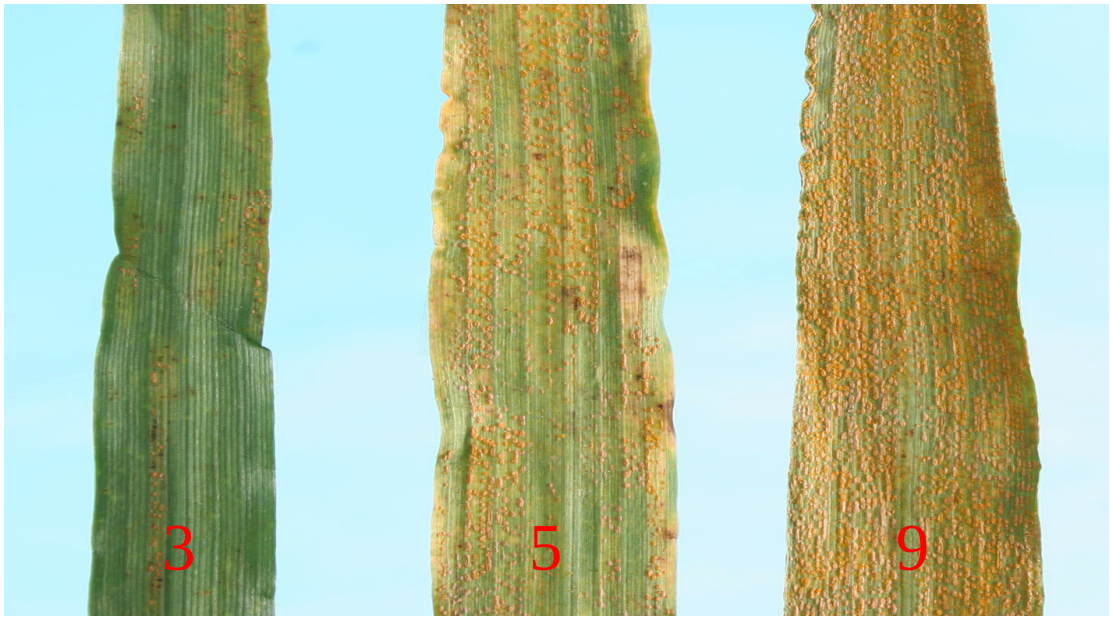


Plate 6.4. Adult plant (flag leaf) responses of parental genotypes (left to right) 'Clipper', 'Sahara 3771' and a susceptible line from 'Clipper'/'Sahara 3771' DH population when assessed under field conditions against Barley Grass Stripe Rust isolate 981549.

6.3.4 Mapping adult plant resistance in Clipper/Sahara 3771 DH population

To assess the response of the 'Clipper'/'Sahara 3771' DH population against *BGYR* at adult plant growth stages, DH lines as well as the parents were sown in the field in 2006 and 2007. Based on disease rating, individual DH lines were classified as either resistant or susceptible. Both parents showed resistance to BGYR under field conditions, indicating the presence of adult plant resistance (APR) in seedling susceptible cultivar 'Clipper', and certain DH lines were susceptible, indicating transgressive segregation (Plate 6.4). Although the disease pressure was relatively higher in 2007, the distribution of resistant and susceptible lines was the same for both years. The population was scored as 104 resistant to 44 susceptible DH lines, conforming to a segregation ratio of 3 resistant : 1 susceptible, expected for segregation of two independent genes (Table 6.7).

Table 6.7. Frequencies of resistant and susceptible lines of 'Clipper'/'Sahara 3771' DH population when screened in the field at adult plant growth stages against Barley Grass Stripe Rust isolate 981549.

DH Population	**Observed frequency**		**Tested ratio (Res : Sus)**	**$\chi 2$**	**P** [a]
	Res	**Sus**			
Clipper/Sahara 3771	104	44	3 : 1	1.77	0.18

$\chi 2$ (1 d.f.) at P = 0.05 is 3.84, [a] P = Probability

All DH lines that were resistant to *BGYR* at seedling growth stages were also resistant in the field, suggesting the seedling resistance gene *Bgyr1*, identified in 'Sahara 3771' was effective at adult plant growth stages. Out of 85 seedling susceptible DH lines (Table 6.3), 40 were resistant at adult plant growth stages and 44 were susceptible at both growth stages. This segregation pattern strongly supported the presence of a single gene conferring APR to *BGYR* in the seedling susceptible cultivar 'Clipper' . The phenotypic response data obtained from the field screening of DH lines in 2007 was used for mapping loci providing resistance to *BGYR* at adult plant growth stages as described by Karakousis *et al*. (2003a). The analysis identified two major QTL on long arm of chromosomes 1 (7H) and 3 (3H) (Fig. 6.2). The QTL located on chromosome 7HL corresponded to the seedling

resistance gene identified in 'Sahara 3771' and the phenotypic marker *Bgyr1* had the maximum LRS value of 42.6 and explained 26% of total phenotypic variance (Table 6.8; Fig. 6.2). The second QTL contributed by 'Clipper' was located on the long arm of chromosome 3H between the marker intervals cdo113 and wg940, with a maximum LRS of 28.1 and explaining 18 % of total phenotypic variance (Table 6.8; Fig. 6.2). This QTL was concluded to correspond to a single APR gene identified in 'Clipper' and was tentatively designated *Bgyr2*.

Table 6.8. Details of two QTL located on chromosome 3HL and 7HL providing resistance at adult plant growth stages to Barley Grass Stripe Rust isolate 981549.

QTL	**Chromosomal location**	**Closest marker**	**LOD**	**Phenotype%** [a]
Bgyr1	7HL	*Bgyr1* (Seedling)	9.2	26
Bgyr2	3HL	CDO113	6.1	18

[a] The portion of explained phenotypic variance

6.4 Discussion

The identification and designation of pathotypes in cereal rust pathogens are based on the response of differential stocks with known resistance genes. These results are used to predict frequency and distribution of existing pathotypes and to identify new pathotypes before they increase to economically important levels. This information is also valuable in identifying effective sources of resistance against the pathotypes relevant to the region of interest (McIntosh *et al.*, 1995). The initial recognition of the distinctiveness of *BGYR* was based on its virulence pattern on the Australian wheat stripe rust differential set. With the exception of partial virulence on 'Chinese 166' carrying *Yr1*, all of the differential genotypes were highly resistant to *BGYR* (Wellings *et al.*, 2000a). However, several other wheat genotypes known to carry *Yr1* were resistant to *BGYR*, suggesting that *BGYR* and *Pst* were distinct biological forms. 'Chinese 166' was also reported to show intermediate infection types against several pathotypes of *Psh* (Stubbs, 1985) and North American *Pst* pathotype *Pst-21* (Chen *et al*., 1993).

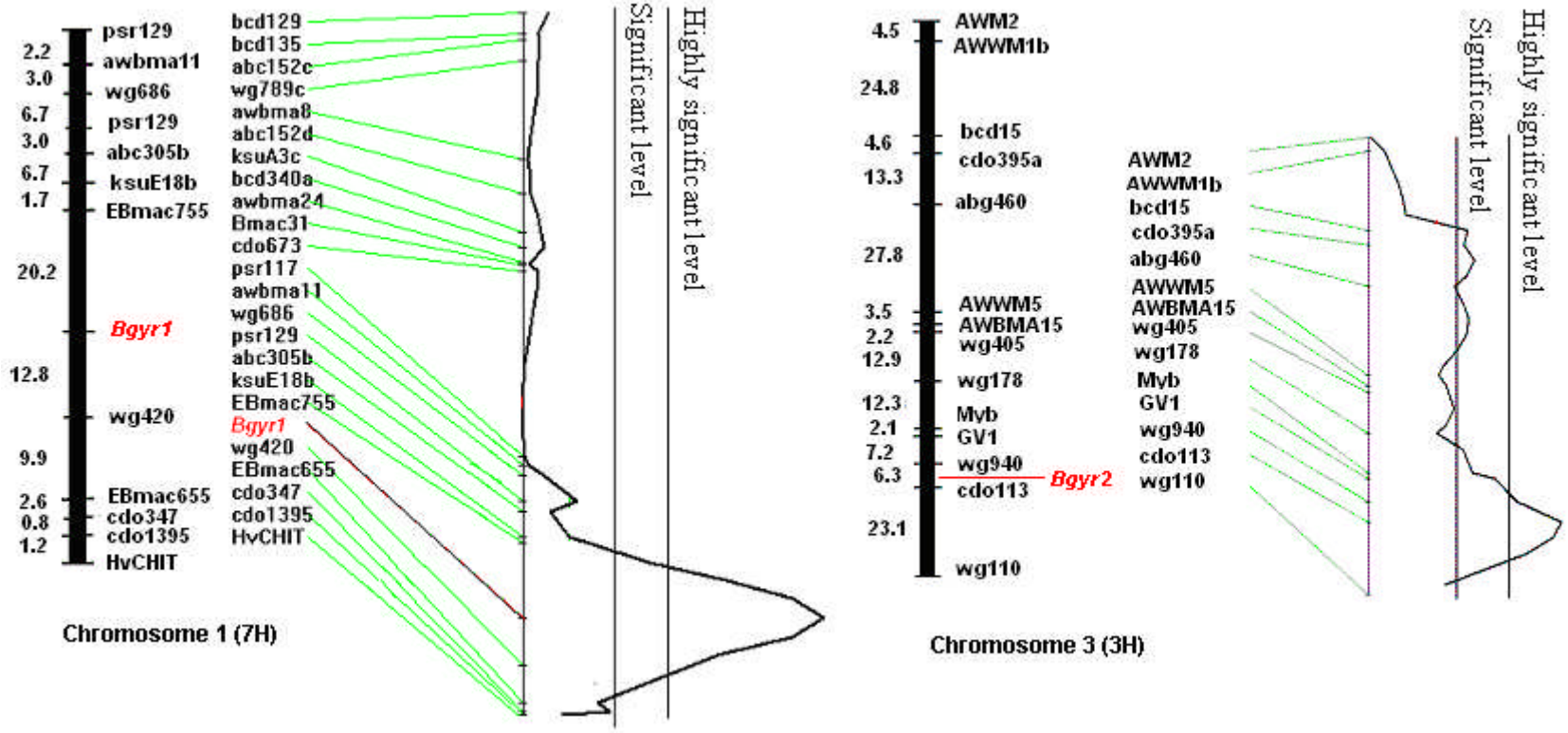


Figure 6.2. Major QTL on chromosome 1 (7H) and chromosome 3 (3H) associated resistance to Barley Grass Stripe Rust at adult plant growth stages in 'Clipper'/'Sahara 3771' doubled haploid population, including thresholds for significant associations (LRS).

It is interesting to note that *Pst-21* was detected in an area where stripe rust was common on wild '*Hordeum* spp' (Line and Qayoum, 1992). DNA polymorphism analyses on North American pathotypes of *P. striiformis* demonstrated close genetic relationships (80%) between all isolates except *Pst-21* (syn. *CDL-21*), which showed only 67% similarity with other isolates (Chen *et al.*, 1993). Based on these results, it was assumed that *Pst-21* had an origin different to the remaining North American *Pst* pathotypes used in the study. Genetic analysis of resistance to *Pst-21* in wheat cultivar 'Lehmi' identified a gene described as *Yr21* (Chen *et al.*, 1995; Pahalawatta and Chen, 2005). 'Lehmi' also carried a single resistance gene against *Psh* pathotypes *Psh-14* and *Psh-48*, and this gene was located 0.3 cM from *Yr21* on chromosome 1B (Pahalawatta and Chen, 2005). The relationship between *Pst-21* and *BGYR* is currently not known. However, molecular studies conducted on Australian isolates of cereal rust pathogens suggested that *BGYR* is genetically distinct from *Pst* and it could represent a new *forma speciales* (Keiper *et al.*, 2003).

BGYR is pathogenic on some Australian barley cultivars, notably 'Skiff' and its derivative 'Tantangara', when tested under greenhouse conditions at seedling growth stages. Under field conditions in Victoria, 'Skiff' and some derivative genotypes recorded significant yield losses (up to 10%) due to severe leaf infection by *BGYR* (Wellings *et al.*, 2000b). These results confirmed the predictions based on greenhouse seedling tests that 'Skiff' and the derivatives could be expected to be vulnerable to the disease. In addition to these two cultivars, the present study also identified six Australian barley cultivars ('Prior', 'Clipper', 'Cutter', 'Keel' and 'Ketch') that showed high ITs in seedling tests to *BGYR*. The pedigrees of these cultivars indicate that they were either selected directly from 'Prior' ('Clipper' and 'Cutter') or from its derivatives ('Keel', 'Ketch', 'Skiff' and 'Tantangara'). 'Prior', released in 1905, was derived from European cultivars 'Archer' or 'Chevalier', and was used widely in Australian barley breeding (Park and Karakousis, 2002). Although 'Skiff' and 'Tantangara' showed high ITs to *BGYR*, single genes providing low levels of resistance to *BGYR* were evident in F_3 populations based on these two cultivars. It was concluded that the intermediate to high IT in these cultivars was conferred by a gene of individual minor effect, that when combined with other genes provided enhanced levels of resistance. Tests of allelism conducted between 'Skiff' and 'Tantangara' indicated that both cultivars carried a common resistance gene to *BGYR*. A recessive seedling resistance gene was identified in the Algerian landrace

'Sahara 3771'. This gene was mapped on chromosome 7HL and was genetically independent of the seedling resistance gene identified in 'Skiff' and 'Tantangara'. It is interesting to note that a large number of recessive genes providing resistance to *Psh* were identified in a range of barley genotypes (Chen and Line, 1999). However, with the exception of a QTL identified at adult plant growth stages (Thomas *et al.*, 1995), no major genes conferring resistance to *P. striiformis* have been mapped to chromosome 7HL in barley. A second gene providing APR to *BGYR* was identified in cultivar 'Clipper'. This gene, tentatively designated as *Bgyr2*, was located on the long arm of chromosome 3H. Toojinda *et al*. (2000) identified a QTL on the long arm of chromosome 3H, providing adult plant resistance to *Psh* in cultivar 'Shyri'. The relationship between the QTL identified in 'Shyri' and *Bgyr2* is currently unknown but is worthy of further investigation.

Based on the results from testing DH populations and those of F_3 populations from 'Skiff'/'Sudan' and 'Tantangara'/'Sudan', it was concluded that the resistance to *BGYR* in cultivars 'Franklin' and 'Tilga' was controlled by two genes and one gene, respectively. The results also suggested that the resistance genes detected in 'Franklin' and 'Tilga' were genetically distinct from the single gene in both 'Skiff' and 'Tantangara'. However their genetic relationship is currently not known. The malting grade Australian barley cultivars 'Stirling' and 'Baudin' each carried two genes conferring resistance to *BGYR*. Additional studies are needed to determine the relationship between the resistance gene identified in these cultivars and those identified in 'Sahara 3771', 'Franklin', 'Tilga' and 'Skiff' or 'Tantangara'. The usefulness of the resistance genes in providing resistance to *Pst* or *Psh* is currently unknown. However, cultivars 'Skiff', 'Tantangara', 'Tilga', 'Baudin', 'Stirling' and 'Franklin' identified as resistant to *BGYR* in the present investigation, were highly susceptible at seedling growth stages to the North American *Psh* races, *race-14* and *race-48* (Wellings, C. R., pers. comm.).

6.5 Conclusion

A set of Australian barley cultivars was screened at seedling growth stages against a variant of *P. striiformis* referred to as *BGYR*. While most Australian barley cultivars were resistant, eight Australian barleys derived from a common lineage were susceptible to *BGYR*. Genetic studies carried out on six Australian barley cultivars and an Algerian landrace indicated that each carried either one or two genes

providing resistance to *BGYR*. Presently, the relationship between most of these resistance genes is unknown. However, the relatively low (1 to 2) number of seedling genes forming the basis of resistance to *BGYR* in the Australian cultivars 'Baudin', 'Stirling', 'Franklin', 'Tilga', 'Skiff' and 'Tantangara' indicates that the *BGYR* pathogen could become a major concern if it acquires virulence for these genes. This has been the experience in wheat against *Pst* and in barley against *Psh*, where resistance conferred by one or two major seedling genes has been often easily overcome by new pathotypes emerging from mutation (Wellings, 2007; Brown *et al*., 2001). Therefore, monitoring the avirulence/virulence patterns of *BGYR* will be important. In this regard, the resistance genes identified in common Australian cultivars could become the basis of differentials to monitor *BGYR*. The seedling susceptible cultivar 'Clipper' showed high levels of APR under field conditions. In the present experiments, the APR in other Australian cultivars could not be assessed due to the presence of major seedling genes providing high levels of resistance to *BGYR*. Current breeding efforts for *BGYR* in Australia are simply directed at avoiding the release of susceptible genotypes (Wellings, C R., pers. comm.). The release of the moderately susceptible cultivar 'Maritime' in 2005 is concerning as it could potentially encourage significant populations of *BGYR* that would be of sufficient magnitude to lead to the evaluation of new pathogenic variants.

CHAPTER VII

General discussion

Breeding for resistance to major diseases is a dynamic area of applied science. It relies on the identification of useful resistance and its incorporation into crop plants to avoid economic losses of yield and quality. Recently, barley leaf rust caused by *P. hordei* has become one of the most important diseases of the barley crop in Australia, due to more intensified barley cultivation and a lack of effective resistance to current pathotypes of *P. hordei* in most cultivated Australian barleys. Consequently, several localised epidemics have been experienced in some barley growing regions of Australia and this has given impetus to develop the genetic resistance as a basis for sustained cultivation of the crop in these regions. Monogenic seedling resistance and polygenic partial resistance are the two resistance sources utilised previously to develop barley cultivars resistant to *P. hordei*. However, a decline in effective seedling resistance genes and difficulty in selecting partial resistance have suggested a need to find new sources of resistance and/or to change the current gene deployment strategy to avoid disease epidemics (Park, 2003).

Six European barley cultivars reputed for their slow rusting characteristics were shown to avoid yield losses under leaf rust epidemic conditions in Australia (Cotterill and Rees, 1993). One of the cultivars, 'Gilbert' selected locally from the slow rusting barley 'Koru', displayed Adult plant resistance (APR) to barley leaf rust (Park, 2003). APR in wheat against stem rust, stripe rust and leaf rust has been widely studied and several APR genes have been characterised. This resistance is valued in wheat for its simple inheritance and widespread durability despite the existence of pathotypes virulent on certain single gene APR (Park and McIntosh, 1994). In an attempt to identify similar form of resistance in barley, a set of 92 barley genotypes composed mainly of European commercial cultivars were assessed for their leaf rust responses at seedling as well as adult plant growth stages. Seedling responses to selected pathotypes of *P. hordei* suggested that most either lacked seedling resistance or possessed one or two known seedling resistance genes that are ineffective in Australia. Only five barley genotypes carried seedling resistances that were effective to all of the pathotypes of *P. hordei* tested, of which four ('Abacus (B)', 'Casino', 'Felicie', 'Monte Cristo') were concluded to carry uncharacterised resistance. These new seedling resistances may be potentially useful in barley

breeding. In field tests, 57 barley genotypes, including several reported to carry slow rusting by Cotterill and Rees (1993) and Parlevliet *et al*. (1980), were identified to carry APR, indicating a close relationship between these two types of resistance. Pedigree information for these barleys indicated that the APR present could have been derived from either '*H. laevigatum*', 'Emir' or from unknown sources.

Genetic studies conducted on 10 barley genotypes suggested that they carried either one gene (i.e. 'Vada', 'Nagrad', 'Gilbert', 'WI3407', 'Ulandra (NT)') or two genes ('Athos', 'Dash', 'Patty', 'Pompadour', 'RAH1995') governing APR to *P. hordei*. The allelism tests between resistant parents indicated that the single APR gene identified in 'Vada' was also present in 'Nagrad', 'Gilbert', 'Athos', 'Patty', 'Pompadour' and 'RAH1995', and that it was distinct from the single APR gene detected in 'WI3407'. Several European barley cultivars derived from 'Vada' or its derivatives and shown to carry APR in the present study were also predicted to carry this gene. Previous studies suggested that cultivar 'Vada' has partial resistance that delays disease development despite being susceptible (Parlevliet, 1979). This kind of resistance was measured using latent period and was reported to be governed by several minor genes (Parlevliet, 1978). The present investigation was unable to confirm these results because 'Vada' displayed high levels of APR under field conditions.

A new technique of bulk segregant analysis (BSA) using hybridisation based DArT markers was used to map leaf rust resistance gene *Rph14*. This gene, identified in an accession of *H. vulgare*, is effective in Australia (Park, 2003). This BSA-DArT method located *Rph14* on the short arm of barley chromosome 2H, and additional studies established close linkage (2.1 to 3.8 cM) with the SSR marker Bmag692. This marker could be useful in marker assisted selection of *Rph14* or in further fine mapping to clone the gene. Because of the small number of samples required and the short processing time, BSA using DArT markers was found to be a very economical and fast means of mapping major genes in barley.

A form of *Puccinia striiformis*, virulent on wild barley grass and several Australian barley cultivars, was detected in Australia in 1998 (Wellings *et al*., 2000a). This pathogen, referred to locally as barley grass stripe rust (*BGYR*), was genetically different from wheat form of stripe rust (Keiper *et al*., 2003). With the exception of 'Clipper', 'Cutter', 'Keel', 'Ketch', 'Maritime', 'Skiff', 'Prior' and 'Tantangra' all Australian barley cultivars tested were resistant to *BGYR* when tested

in the greenhouse at seedling growth stages. Genetic studies carried out on six commonly grown Australian barley cultivars and an Algerian landrace indicated that they carried either 1 or 2 seedling resistance genes to *BGYR*. However, the diversity of resistance genes present in Australian barley cultivars is not known. A single seedling resistance gene, *Bgyr1*, identified in 'Sahara 3771' and a single APR gene, *Bgyr2*, detected in 'Clipper', were mapped to the long arms of chromosomes 7H and 3H, respectively. These genes, together with the genes identified in Australian barley cultivars, could be useful in monitoring pathogenic variability in *BGYR* and to examine potential relationships with resistance genes in barley to other forms of *Puccinia striiformis*.

The present study identified potentially new sources of APR to *P. hordei* in barley and investigated its inheritance under Australian conditions. The studies were also conducted to find new sources of seedling resistance and to find closely linked markers for seedling resistance gene *Rph14* that could be used in marker assisted selection. These findings are the initial steps for breeding barleys with durable resistance to leaf rust. The potential impact of *BGYR* was assessed by analysing genetic resistance present in selected Australian barley cultivars. Because the resistance in several Australian barleys was determined by 1 or 2 seedling resistance genes, it will be important to monitor pathogenicity in *BGYR* in order to detect any potential changes before they threaten the commercial barley crop. In this context, the resistance genes identified in the present studies could serve as differential genotypes. Based on these results, the following future directions are suggested to improve the use of the resistance sources identified during the study:

1) Genetic analyses of APR to *P. hordei* in 47 barley genotypes and determination of their genetic relationship with the single APR genes identified in 'Vada' and 'WI3407'
2) Mapping the APR genes in 'Vada' and 'WI3407'
3) Characterisation of potentially new seedling resistance to leaf rust detected in cultivars 'Abacus (B)', 'Casino', 'Felicie', Monte Cristo and 'Roland'
4) Tests of allelism between the seedling genes providing resistance to *BGYR*

References

Agrama, H. A., Dahleen, L., Wentz, M., Jin, Y. and Steffenson, B. (2004). Molecular mapping of the crown rust resistance gene *Rpc1* in barley. Phytopathol. **94**: 858-861.

Allard, R. W. (1960). Principles of Plant Breeding. John Wiley and Sons, Inc., New York.

Anikster, Y. (1982). Alternate hosts of *Puccinia hordei*. Phytopathol. **72**: 733-735.

Anikster, Y. (1984). The *formae speciales*. In: The Cereal Rusts Vol.1 (Bushnell, W. R., and Roelfs, A. P., eds.), pp. 115-130. Academic Press, Inc., New York.

Anikster, Y., Moseman, J. G. and Wahl, I. (1976). Parasite specialization of *Puccinia hordei* Otth. and sources of resistance. Proc. 3rd Intl. Barley Genetics Symposium, Garching, Germany.

Aradhya, M. K., Chan, H. M. and Parfitt, D. E. (2001). Genetic variability in the pistachio late blight fungus, *Alternaria alternata*. Mycol. Res. **105**: 300-306.

Arnst, B. J., Martens, J. W., Wright, G. M., Burnett, P. A. and Sanderson, F. R. (1979). Incidence, importance, and virulence of *Puccinia hordei* on barley in New Zealand. Ann. Appl. Biol. **92**: 185-190.

Backes, G., Madsen, L. H., Jaiser, H., Stougaard, J., Herz, M., Mohler, V. and Jahoor, A. (2003). Localisation of genes for resistance against *Blumeria graminis* f. sp. *hordei* and *Puccinia graminis* in a cross between a barley cultivar and a wild barley (*Hordeum vulgare* ssp. *spontaneum*) line. Theor. Appl. Genet. **106**: 353-362.

Backes, G., Schwarz, G., Wenzel, G. and Jahoor, A. (1996). Comparision between QTL analyses on powdery mildew resistance in barley based on detached primary leaves and on field data. Plant Breed. **115**: 419-421.

Barcellos, A. L. Roelfs, A. P. and de Moraes-Fernandes, M. I. B. (2000). Inheritance of adult plant resistance in the Brazilian wheat cultivar Toropi. Plant Dis. **84**: 90-93.

Bard, A., Muller, K., Schafer-Pregl, R., Rabey, El. H., Effgen, S., Ibrahim, H. H., Pozzi, C., Rohde, W. and Salamini, F. (2000). On the origin and domestication history of barley (*Hordeum vulgare*). Mol. Biol. Evol. **17**: 499-510.

Basten, C. J., Weir, B. S. and Zeng, Z. B. (1999). QTL Cartographer, version 1.13. Raleigh, North Carolina State University.

Biffen, R. H. (1905). Mendel's law of inheritance and wheat breeding. J. Agri. Sci. **1**: 4-48.

Borlaug, N. E. (1953). New approaches to the breeding of wheat varieties resistant to *Puccinia graminis tritici*. Phytopathol. **43**: 467.

Borovkova, I. G., Jin, Y., and Steffenson, B. J. (1998). Chromosomal location and genetic relationship of leaf rust resistance genes *Rph9* and *Rph12* in barley. Phytopathol. **88**: 76-80.

Borovkova, I. G., Jin, Y., Steffenson, B. J., Kilian, A., Blake, T. K. and Kleinhofs, A. (1997). Identification and mapping of a leaf rust resistance gene in barley line Q21861. Genome **40**: 236-241.

Botstein, D., White, R. L., Skolnick, M. and Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. **32**:314-331.

Browder, L. E. (1971). Pathogenic specialization in cereal rust fungi, especially *Puccinia recondita* f. sp. *tritici*, concepts, methods of study, and application. Agric. Res. Ser. Tech. Bull. No. 1432 (USDA: Washington DC).

Brookes, A. J. (1999). The essence of SNPs. Gene **234**:177-186.

Brown, W. M., Hill, J. P. and Velasco, V. R. (2001). Barley yellow rust in North America. Ann. Rev. Phytopathol. **39**:367-384.

Brückner, F. (1970). Varietal resistance of spring barley to leaf rust (*Puccinia hordei* Otth.) in Czechoslovakia (in Czech, English Abstr.). Genet. Slecht. **6**: 143-151.

Brunner, S., Keller, B. and Feuillet, C. (2000). Molecular mapping of the *Rph7*.g leaf rust resistance gene in barley (*Hordeum vulgare* L.). Theor. Appl. Genet. **101**: 783-788.

Burnham, C. R. and Hagberg, A. (1956). Cytogenetic notes on chromosomal interchanges in barley. Hereditas **42**: 467-482.

Caranta, C., Letesure, V. and Palloix, A. (1997). Polygenic resistance of pepper to poly viruses consists of a combination of isolate specific and broad- spectrum quantitative trait loci. Mol. Plant Microbe Interact. **10**: 372-378.

Chelkowski, J., Tyrka, M. and Sobkiewiez, A. (2003). Resistance genes in barley and their identification with molecular markers. J. Appl. Genet. **44**: 291-309.

Chen, X.M., Line, R.F. and Leung, H. (1993). Relationship between virulence variation and DNA polymorphism in *Puccinia striiformis*. Phytopathol. **83**: 1489-1497.

Chen, X.M., Line, R.F. and Jones, S.S. (1995). Location of genes for stripe rust in spring wheat cultivars Compair, Fielder, Lee and Lehmi and interactions of aneuploid wheats with races of *Puccinia striiformis*. . Phytopathol. **85**: 375-381.

Chen, X.M. and Line, R.F. (1999). Recessive genes for resistance to races of *Puccinia striiformis* f. sp. *hordei* in barley. Phytopathol. **89**: 226-232.

Chicaiza, O., Franckowiak, J. D. and Steffenson, B. J. (1996). New sources of resistance to leaf rust in barley. Proc. 5th Int. Oat Conf. & 7th Int. Barley Genet. Symp., University of Saskatchewan, Saskatoon.

Clifford, B. C. (1977). Monitoring virulence in *Puccinia hordei*: A proposal for the choice of host genotypes and survey procedures. Cereal Rusts Bull. **5**: 34-38.

Clifford, B. C. (1985). Barley leaf rust. In: The Cereal Rusts (Roelfs, A. P., and Bushnell, W. R., eds.), pp. 173-205. Academic Press, Orlando, Florida, USA.

Clifford, B. C. (1992). Application of the octal/binary notation system to virulence nomenclature in *Puccinia hordei*, the cause of brown rust of barley. Cereal Rusts and Powdery Mildews Bull. **20**: 33-37.

Clifford, B. C. and Udeogalanya, A. C. C. (1976). Hypersensitive resistance of barley to brown rust (*Puccinia hordei* Otth.). Proc. 4th Eur. Mediterr. Cereal Rusts Conf., 27-29.

Cobb, N. A. (1892). Contributions to an economic knowledge of the Australian rusts (*Uredineae*). Agr. Gaz. N. S. W. **3**: 60-68.

Collins, N., Park, R., Spielmeyer, J. and Pryor, A. J. (2001). Resistance gene analogs in barley and their relationship to rust resistance genes. Genome. **44**: 375-381.

Cotterill, P. J. and Rees, R. G. (1993). Evaluation of European slow-rusting barleys in Queensland. Trends Agrochem. Cultivars **14**: 166-167.

Cotterill, P. J., Park, R. F. and Rees, R. G. (1995). Pathogenic specialization of *Puccinia hordei* Otth. in Australia, 1966-1990. Aus. J. Agric. Res. **46**: 127-134.

Cotterill, P. J., Rees, R. G. and Platz, G. J. (1994). Response of Australian barley cultivars to leaf rust (*Puccinia hordei*). Aus. J. Exp. Agric. **34**: 783-788.

Cotterill, P. J., Rees, R. G., Platz, G. J. and Dill-Macky, R. (1992). Effects of leaf rust on selected Australian barleys. Aus. J. Exp. Agric. **32**: 747-751.

Critopoulos, P. (1956). Perpetuation of the brown rust of barley in Attica. Mycol. **48**: 596-600.

Cromey, M. G. and Villjanen-Rollinson, S. L. H. (1995). Virulence of *Puccinia hordei* on barley in New Zealand from 1990 to 1993. N. Z. J. Crop Hortic. Sci. **23**: 115-119.

Crute, I. R. and Pink, D. A. C. (1996). Genetics and utilization of pathogen resistance in plants. Plant Cell **8**: 1747-1755.

Cummins, G. B. and Stevenson, J. A. (1956). A checklist of North American rust fungi (*Uredinales*). Plant Dis. Rep. Suppl. **240**:109-193.

Datta, K., Baisakh, N., Maung, T. K., Tu, J. and Datta, S. K. (2002). Pyramiding transgenes for multiple resistance in rice against bacterial blight, yellow stem borer and sheath blight. Theor. Appl. Genet. **106**:1-8.

Deshmukh, M. J. and Howard, H. W. (1956). Field resistance to potato blight (*Phytopthora infestans*). Nature **117**: 794-795.

Devaux, P. (1988). Comparison of anther culture and *Hordeum bulbosum* method for the production of doubled haploids in winter barley. Plant Breed. **100**: 181-187.

Dillard, M. W. and Brown, A. R. (1969). Inheritance of reaction to race 8 of *Puccinia hordei* Otth. in two barley crosses. Crop Sci. **9**: 677-678.

Dill-Macky, R., Rees, R. G., Johnston, R. P., Platz, G. J. and Mayne, A. (1989). Stem and leaf rusts of barley. Queensland Wheat Research Institute Biennial Report 1984-86, Qeensland Department of Primary Industries, Toowoomba.

Doyle, J. and Doyle, J. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. **19**: 11-15.

Dreiseitl, A. and Steffenson, B. J. (2000). Postulation of leaf-rust resistance genes in Czech and Slovak barley cultivars and breeding lines. Plant Breed. **119**: 211-214.

Drijepondt, S. C., Pretorius, Z. A. and Rijkenberg, F. H. J. (1991). Expression of two wheat leaf rust resistance gene combinations involving *Lr*34. Plant Dis. **75**: 526-528.

Dros, J. (1957). The creation and maintenance of two spring barley varieties. Euphytica **6**: 45-48.

Dubin, H. J. and Stubbs, R. W. (1986). Epidemic spread of barley stripe rust in S. A. Plant Dis. **70**:141-144.

Dyck, P. L. (1987). The association of a gene for leaf rust resistance with the chromosome 7D suppressor of stem rust resistance in common wheat. Genome **29**: 467-469.

Dyck, P. L., Samborski, D. J. and Andersen, R. G. (1966). Inheritance of adult plant leaf rust resistance derived from the common wheat varieties Exchange and Frontana. Can. J. Genet. Cytol. **8**: 665-671.

Eriksson, J. and Hanning, E. (1894). Ueber die Specialisierung des Parasitismus bei den Getreiderostpilzen. Ber. Dtsch. Bot. Ges. **12**: 292-331.

Falconer, D. S. and Mackey, T. F. C. (1996). Introduction to quantitative genetics. Longman Group Ltd., Edinburgh.

FAO (2004). http://faostat.fao.org/site/340/default.aspx. 16/05/2005

Fetch, T. G., Jr., Steffenson, B. J. and Jin, Y. (1998). Worldwide virulence of *Puccinia hordei* on barley. Phytopathol. **88**: S28.

Feuerstein, U., Brown, A. H. D. and Burdon, J. J. (1990). Linkage of rust resistance genes from wild barley (*Hordeum spontaneum*) with isozyme markers. Plant Breed. **104**: 318-324.

Flor, H. H. (1956). The complementary genic systems in flax and flax rust. Adv. Genet. **8**: 29-54.

Franckowiak, J. D., Jin, Y. and Steffenson, B. J. (1997). Recommended allele symbols for leaf rust resistance genes in barley. Barley Genet. Newslett. **27**: 36-44.

Frecha, J. H. (1970). Inheritance of the resistance to *Puccinia hordei* Otth. in barley. Biol. Genet. (Engl. Ed.) **7**: 1-8.

Frecha, J. H. (1971). Inheritance of the resistance to *Puccinia hordei* Otth. in barley. Inf. Tec. Estac. Exp. Reg. Agropecu. Pergamino. **105**: 38-42.

Gassner, G. and Straib, W. (1932). Die bestimmung der biologischen rassen des weizengelbrostes (*Puccinia glumarum* f. sp. *tritici* (Schmidt.) Erikss. und Henn.). Arbeiten der Biologischen Reichsanstalt fur Land und Forstwirtschaft, Berlin **20**: 141-163.

Geffroy, V., Sevignac, M., De Oliveira, J. C. F., Fouilloux, G., Skroch, P., Thoquet, P., Gepts, P., Langin, T. and Dron, M. (2000). Inheritance of partial resistance against *Colletotrichum lindemuthianum* in *Phaseolus vulgaris* and co-localisation of quantitative trait loci with genes involved in specific resistance. Mol. Plant Microbe Interact. **13**: 287-296.

Genger, R. K., Nesbitt, K., Brown, A. H. D., Abbott, D. C. and Burdon, J. J. (2005). A novel barley scald resistance gene: genetic mapping of the *Rrs15* scald resistance gene derived from wild barley, *Hordeum vulgare* ssp. *spontaneum*. Plant Breed. **124**: 137-141.

Giese, H., Holm-Jensen, A. G., Jensen, H. P. and Jensen, J. (1993). Localisation of the Laevigatum powdery mildew resistance gene to barley chromosome 2 by the use of RFLP markers. Theor. Appl. Genet. **85**: 897-900.

Gilmour, J. (1973). Octal notation for designating physiologic races of plant pathogens. Nature (London) **242**: 620.

Golan, T., Anikster, Y., Moseman, J. G. and Wahl, I. (1978). A new virulent strain of *Puccinia hordei*. Euphytica **27**: 185-189.

Golegaonkar, P. G., Park, R. F. and Singh, D. (2006). Adult plant resistance to *Puccinia hordei* in barley. Proc. Intl. Plant Breed. Symp., Mexico City, Mexico.

Graner, A., Streng, S., Drescher, A., Jin, Y., Borovkova, I. and Steffenson, B. J. (2000). Molecular mapping of the leaf rust resistance gene *Rph7* in barley. Plant Breed. **119**: 389-392.

Griffey, C. A., Das, M. K., Baldwin, R. E. and Waldenmaier, C. M. (1994). Yield losses in winter barley resulting from a new race of *Puccinia hordei* in North America. Plant Dis. **78**: 256-260.

Haahr, V., Skou, J. P. and Jensen, H. P. (1989). Inheritance of resistance to barley leaf stripe *(Drechslera graminea).* Vortr. Pflanzenzüchtg. 15. Abstract no. **3-15**: 2.

Haley, C. S. and Knott, S. A. (1992). A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity **69**: 315-324.

Harlan, H. V. and Pope, M. N. (1922). The use and value of back-crosses in small grain breeding. J. Heredity **13**: 319-322.

Harlan, J. R. (1976). Barley. In: Evolution of Crop Plants (Simmonds, N. W. ed.), pp. 93-98. Longman Press, London, UK.

Harlan, J. R. (1995). The Living Fields: Our Agricultural Heritage. Cambridge University Press, Cambridge, UK.

Heath, M. C. (1998). Apoptosis, programmed cell death and the hypersensitive response. Eur. J. Plant Pathol. **104**: 117-124.

Henderson, M. T. (1945). Studies of sources of resistance and inheritance of reaction to leaf rust *Puccinia anomala* Rostr. in barley. Ph.D. Thesis, University of Minnesota, Minneapolis.

Hittalmani, S., Parco, A., Mew, T. V., Zeigler, R. S. and Huang, N. (2000). Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theor. Appl. Genet. **100**: 1121-1128.

Hylander, N. Jorstad, I. and Nannfeldt, J. A. (1953). Enameratio uredinearum scandinavicarum, Opera. Botanica **1**: 1-102.

Ivandic, V., Walther, U. and Graner, A. (1998). Molecular mapping of a new gene in wild barley conferring complete resistance to leaf rust (*Puccinia hordei* Otth). Theor. Appl. Genet. **97**: 1235-1239.

Jansen, R. C. (1993). Interval mapping of multiple quantitative trait loci. Genetics **135**: 205-211.

Jensen, H. P. and Jørgensen, J. H. (1991). Genetics of '*Laevigatum*' resistance and virulence in barley and powdery mildew. Barley Genet. Newslett. **21**: 34-37.

Jenkins, J. E. E., Melville, S. C. and Jemmett, J. L. (1972). The effect of fungicides on leaf diseases and on yield in spring barley in south-west England. Plant Pathol. **21**: 49-58.

Jensen, N. F. (1952). Intervarietal diversification in oat breeding. Agro. J. **44**: 30-34.

Jin, Y., Cui, G. H., Steffenson, B. J. and Franckowiak, J. D. (1996). New leaf rust resistance genes in barley and their allelic and linkage relationships with other *Rph* genes. Phytopathol. **86**: 887-890.

Jin, Y., Statler, J. D., Franckowiak, J. D. and Steffenson, B. J. (1993). Linkage between leaf rust resistance genes and morphological markers in barley. Phytopathol. **83**: 230-233.

Jin, Y. and Steffenson, B. J. (1994). Inheritance of resistance to *Puccinia hordei* in cultivated and wild barley. J. Heredity **85**: 451-454.

Jin, Y., Steffenson, B. J. and Bockelman, H. E. (1995). Evaluation of cultivated and wild barley for resistance to pathotypes of *Puccinia hordei* with wide virulence. Genet. Resour. Crop Evol. **42**: 1-6.

Johnson, R. (1968). The genetics of resistance of some barley varieties to *Puccinia hordei.* Proc. Eur. Meditter. Cereal Rust Conf., Oeiras, Portugal.

Johnson, R. (1970). The genetics of resistance of some barley varieties to *Puccinia hordei.* Proc. 2nd Eur. Mediterr. Cereal Rusts Conf.

Johnson, R. (1978). Practical breeding for durable resistance to rust diseases in self pollinating cereals. Euphytica **27**: 529-540.

Johnson, R. (1981). Durable resistance, defination of, genetic control, and attainment in plant breeding. Phytopathol. **71**: 567-568.

Johnson, R. (1984). A critical analysis of durable resistance. Ann. Rev. Phytopathol. **22**: 309-330.

Jørgensen, J. H. (1992). Discovery, characterization and exploitation of *Mlo* powdery mildew resistance in barley. Euphytica **63**: 141-152.

Jørgensen, J. H. (1983). Durability of barley powdery mildew resistance genes in Denmark 1963-1980. In: Durable Resistance in Crops (Lamberti, F., Waller, J. M., Van der Graff, N. A., eds.), Plenum Publication. New York, pp. 397-399.

Karakousis, A., Gustafson, J. P., Chalmers, K. J., Barr, A. R. and Langridge, P. (2003a). A consensus map of barley integrating SSR, RFLP, and AFLP markers. Aust. J. Agril. Res. **54**: 1173-1185.

Karakousis, A., Gustafson, J. P., Chalmers, K. J., Barr, A. R. and Langridge, P. (2003b). A consensus map of barley integrating SSR, RFLP, and AFLP markers. Aust. J. Agril. Res. **54**: 1173-1185.

Keiper, F. J., Hayden, M. J., Park, R. F. and Wellings, C. R. (2003). Molecular genetic variability of Australian isolates of five cereal rust pathogens. Mycol. Res. **107**: 545-556.

Keller, M., Keller, B., Schachermayr, G., Winzeler, M., Schmid, J. E., Stamp, P. and Messmer, M. M. (1999). Quantitative trait loci for resistance against powdery mildew in a segregating wheat x spelt population. Theor. Appl. Genet. **98**: 903-912.

Kicherer, S., Backes, G., Walther, U. and Jahoor, A. (2000). Localising QTLs for leaf rust resistance and agronomic traits in barley (*Hordeum vulgare* L.). Theor. Appl. Genet. **100**: 881-888.

King, J. E. (1977). Surveys of foliar diseases of spring barleys in England and Wales, 1972-75. Plant Pathol. **26**: 21-29.

King, J. E. and Polley, R. W. (1976). Observations on the epidemiology and effect on grain yield of brown rust in spring barley. Plant Pathol. **25**: 63-73.

Kislev, M. E. (1982). Stem rust of wheat 3300 years old found in Israel. Science **216**: 993-994.

Kolmer, J. A., Dyck, P. L. and Roelfs, A. P. (1991). An appraisal of stem and leaf rust resistance in North American hard red spring wheats and probability of multiple mutations to virulence in populations of cereal rust fungi. Phytopathol. **81**: 237-239.

Kota, R., Spielmeyer, W., McIntosh, R. A. and Lagudah, E. S. (2006). Fine genetic mapping fails to dissociate durable stem rust resistance gene Sr2 from pseudo-black chaff in common wheat (*Triticum aestivum* L.) Theor. Appl. Genet. **112**: 492-499.

Kuenzel, G., Korzun, L. and Meister, A. (2000). Cytologically integrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints. Genetics **154**: 397-412.

Lander, E. S. and Botstein, D. (1989). Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics **121**: 185-199.

Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E. and Newburg, L. (1987). MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics **1**: 174-181.

Langridge, P. and Barr, A. R. (2003). Preface. Aust. J. Agril. Res. **54**: i-iv.

Laundon, G. F. (1973). Uredinales. In: The Fungi (Ainsworth, G. C., Sparrow, F. K., and Sussman, A. S., eds.), pp. 247-279. Academic Press, New York.

Levine, M. N. and Cherewick, W. J. (1952). Studies on dwarf leaf rust of barley. U.S., Dept. Agric., Tech. Bull. **1056**: 1-17.

Levine, M. N. and Cherewick, W. J. (1956). Greenhouse and field plot studies on varietals reactions to barley leaf rust. Can. J. Agric. Sci. **36**: 127-143.

Li, Z. K., Luo, L. J., Mei, H. W., Paterson, A. H., Zhao, X. H., Zhong, D. B., Wang, Y. P., Yu, X. Q., Zhu, L., Tabein, R., Stansel, J. W. and Ying, C. S. (1999). A "defeated" rice resistance gene acts as a QTL against a virulent strain of *Xanthomonous oryzae*. Mol. Gen. Genet. **261**: 58-63.

Lim, L. G. and Gaunt, R. E. (1986). The effect of powdery mildew (*Erysiphe graminis* f. sp. *hordei*) and leaf rust (*Puccinia hordei*) on spring barley in New Zealand. II. Apical development and yield potential. Plant Pathol. **35**: 54-60.

Line, R. F. (2002). Stripe rust of wheat and barley in North America: a retrospective historical review. Ann. Rev. Phytopathol. **40**: 75-118.

Lincoln, S., Daly, M. and Lander, E. (1993). Mapping genes controlling quantitative traits with MAPMAKER/QTL Version 1.1: A tutorial and reference manual. Cambridge MA, Whitehead Institute for Biomedical Research.

Line, R. F. and Qayoum, A. (1991). Virulence, aggressiveness, evolution and distribution of races of *Puccinia strriformis* (the cause of stripe rust of wheat) in North America 1968-87. Agric. Res. Ser. Tech. Bull. No. 1788. Pullman, WA:USDA.

Liu, Z. W., Biyashev, R. M. and Maroof, M. A. S. (1996). Development of simple sequence repeat DNA markers and their integration into a barley linkage map. Theor. Appl. Genet. **93**: 869-876.

Love, H. H. (1927). A programme for selecting and testing small grains in successive generations for hybridisation. Amer. Soc. of Agro. J. **19**: 705-712.

Lucas, J. A. (1998). Plant Pathology and Plant Pathogens, 3rd ed. Blackwell Publishing, London.

Luig, N. H. (1983). A survey of virulence genes in wheat stem rust, *Puccinia graminis* f. sp. *tritici*. Adv. Plant Breed., Supplement 11 to J. Plant Breed.

Luig, N. H. (1985). Epidemiology in Australia and New Zealand. In: The Cereal Rusts Vol II (Roelfs, A. P., and Bushnell, W. R., eds.), pp. 301-328. Academic Press, Orlando.

Luig, N. H. and Watson, I. A. (1972). The role of wild and cultivated grasses in the hybridisation of *formae speciales* of *Puccinia graminis*. Aus. J. Biol. Sci. **25**: 335-342.

Mackey, D., Holt, B., Wiig, A. and Dangl, J. (2002) RIN4 interacts with Pseudomonas syringae type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis. Cell **108**: 743-754.

Mammadov, J. A., Zwonitzer, J. C., Biyashev, R. M., Griffey, C. A., Jin, Y., Steffenson, B. J. and Maroof, M. A. S. (2003). Molecular mapping of leaf rust resistance gene *Rph5* in barley. Crop Sci. **43**: 388-393.

Manisterski, J. (1989). Physiologic specialization of *Puccinia hordei* in Israel from 1983 to 1985. Plant Dis. **73**: 123-135.

Manly, K. F., Cudmore, Jr. R. H. and Meer, J. M. (2001). Map Manager QTX, cross-platform software for genetic mapping. Mammalian Genome **12**: 930-932.

Martineaz, O. and Curnow, R. N. (1992). Estimating the locations and the sizes of the effects of quantitative trait loci using flanking markers. Theor. Appl. Genet. **85**: 480-488.

Masojc, P. (2002). The application of molecular markers in the process of selection. Cell. Mol. Biol. Lett. **7**: 499-509.

Mathre, D. E. (1982). Compendium of barley diseases. Proc. Am. Phytopathol. Soc. 32-34.

McIntosh, R. A. and Brown, G. N. (1997). Anticipatory breeding for resistance to rust diseases in wheat. Ann. Rev. Phytopathol. **35**: 311-326.

McIntosh, R. A., Wellings, C. R. and Park, R. F. (1995). Wheat Rusts: An Atlas of Resistance Genes. CSIRO Australia, Melbourne.

McNeal, F. H., Konzak, C. F., Smith, E. P., Tate, W. S. and Russell, T. S. (1971). A uniform system for recording and processing cereal research data. Agri. Res. Ser. Bull. (U. S. State Dept. Agri. Washington) **34**: 121.

Melville, S. C., Griffin, G. W. and Jemmett, J. L. (1976). Effects of fungicide spraying on brown rust and yield in spring barley. Plant Pathol. **25**: 99-107.

Mendel, G. (1865). Experiments in plant hybridisation. In: Classic Papers in Genetics (Peters, J. A., ed.) Prentice-Hall: Englewood Cliffs, N.J., USA.

Michelmore, R. W., Paran, I. and Kesseli, R. V. (1991). Identification of markers linked to disease resistance genes by bulk segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating population. Proc. Natl. Acad. Sci. USA. **88**: 9828-9832.

Micke, A., Donini, B. and Maluszynski, M. (1990). Induced mutations for crop improvement. Mut. Breed. Rev. **7**: 1-41.

Mohler, V. and Singrun, C. (2004). General considerations: marker-assisted selection. In: Biotechnology in Agriculture and Forestry. Vol. 55. Molecular Marker Systems in Plant Breeding and Crop Improvement (Lorz, H. and Wenzel, G. eds.), pp. 305-317. Springer-Verlag, Berlin, Heidelberg.

Moseman, J. G. (1972). Reports on genes for resistance to pests. Barley Genet. Newslett. **2**: 145-147.

Moseman, J. G. and Greeley, L. W. (1965). New pathogenic strains of *Puccinia hordei* among physiological races identified in United States from 1959 through 1964. Plant Dis. Rep. **49**: 575-578.

Moseman, J. G. and Reid, D. A. (1961). Linkage relationship of genes conditioning resistance to leaf rust and powdery mildew in Franger barley. Crop Sci. **1**: 425-427.

Moseman, J. G. and Roan, C. W. (1959). Physiologic races of barley leaf rust (*Puccinia hordei*) isolated in the United States from 1956 through 1958. Plant Dis. Rep. **43**: 1000-1003.

Neervoort, W. J. and Parlevliet, J. E. (1978). Partial resistance of barley to leaf rust, *Puccinia hordei* V. Analysis of components of partial resistance in eight barley cultivars. Euphytica **27**: 33-39.

Newton, M., Peturson, B. and Meredith, W. O. S. (1945). The effect of leaf rust of barley on the yield and quality of barley varieties. Can. J. Res., Sect. C **23**: 212-218.

Niks, R. E., Walther, U., Jaiser, H., Martinez, F., Rubiales, D., Andersen, O., Flath, K., Gymer, P., Heinrichs, F., Jonsson, R., Kuntze, L., Rasmussen, M. and Richter, E. (2000). Resistance against barley leaf rust (*Puccinia hordei*) in West-European spring barley germplasm. Agronomie **20**: 769-782.

Nover, I. and Lehmann, C. O. (1974). Resistenzeigenschaften im Gerstenund Weizensortiment Gatersleben 18. Prufung von Sommergersten auf ihr verhalten gegen Zwergrost (*Puccinia hordei* Otth.). Kulturpflanze **22**: 25-43.

Ott, J. (1985). Analysis of Human Genetic Linkage. The John Hopkins Press Ltd., London, p. 197.

Pahalawatta, V. and Chen, X.M. (2005). Genetic analysis and molecular mapping of wheat genes conferring resistance to the wheat stripe rust and barley stripe rust pathogens. Phytopathol. **95**: 427-432.

Park, R. F. (2003). Pathogenic specialization and pathotype distribution of *Puccinia hordei* in Australia, 1992 to 2001. Plant Dis. **87**: 1311-1316.

Park, R. F. (2000). Rust Fungi. Encyclopedia of Microbiology. **4**: 195-211.

Park, R. F. and Karakousis, A. (2002). Characterization and mapping of gene *Rph19* conferring resistance to *Puccinia hordei* in the cultivar 'Reka 1' and several Australian barleys. Plant Breed. **121**: 232-236.

Park, R. F. and McIntosh, R. A. (1994). Studies of single gene adult plant resistances to *Puccinia recondita* f. sp. *tritici* in wheat. N. Z. J. Crop Hortic. Sci. **22**: 151-158.

Park, R. F., Poulsen, D., Barr, A. R., Cakir, M., Moody, D. B., Raman, H. and Read, B. J. (2003). Mapping genes for resistance to *Puccinia hordei* in barley. Aus. J. Agric. Res. **54**: 1323-1333.

Parlevliet, J. E. (1976a). The genetics of seedling resistance to leaf rust, *Puccinia hordei* Otth. in some spring barley cultivars. Euphytica **25**: 249-254.

Parlevliet, J. E. (1976b). Partial resistance of barley to leaf rust, *Puccinia hordei*. III. The inheritance of the host plant effect on latent period in four cultivars. Euphytica **25**: 241-248.

Parlevliet, J. E. (1979). Components of resistance that reduce the rate of epidemic development. Ann. Rev. Phytopathol. **17**: 203-222.

Parlevliet, J. E. (1978). Further evidence of polygenic inheritance of partial resistance in barley to leaf rust, *Puccinia hordei*. Euphytica **27**: 369-379.

Parlevliet, J. E. (1983). Race specific resistance and cultivar-specific virulence in the barley-leaf rust pathosystem and their consequences for the breeding of leaf rust resistant barley. Euphytica **32**: 367-375.

Parlevliet, J. E. (1986). Pleiotropic association of infection frequency and latent period of two barley cultivars partially resistant to barley leaf rust. Euphytica **35**: 267-272.

Parlevliet, J. E. and Kievit, C. (1986). Development of barley leaf rust, *Puccinia hordei*, infections in barley. I. Effect of partial resistance and plant stage. Euphytica **35**: 953-959.

Parlevliet, J. E. and Kupier, H. J. (1985). Accumulating polygenes for partial resistance in barley to barley leaf rust, *Puccinia hordei*. I. Selection for increased latent periods. Euphytica **34**: 7-13.

Parlevliet, J. E., Lindhout, W. H., Ommeren, A. V. and Kuiper, H. J. (1980). Level of partial resistance to leaf rust, *Puccinia hordei*, in West-European barley and how to select for it. Euphytica **29**: 1-8.

Parlevliet, J. E., Van Der Beek, J. G. and Pieters, R. (1981). Presence in Morocco of brown rust, *Puccinia hordei*, with a wide range of virulence to barley. Cereal Rusts Bull. **9**: 3-8.

Parlevliet, J. E. and van Ommerson, A. (1975). Partial resistance of barley to leaf rust, *Puccinia hordei*. II. Relationship between field trials, micro plot tests and latent period. Euphytica **24**: 293-303.

Parlevliet, J. E., Lindhout, W. H., Ommeren, A. V. and Kuiper, H. J. (1980). Level of partial resistance to leaf rust, *Puccinia hordei*, in West-European barley and how to select for it. Euphytica **29**: 1-8.

Peterson, R. F., Campbell, A. B. and Hannah, A. E. (1948). A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. Can. J. Res., Sect. **26**: 496-500.

Pickering, R. A., Hill, A. M. and Kyanst, R. G. (1997). Characterization by RFLP analysis and genomic in situ hybridization of a recombinant and a monosomic plant derived from a *Hordeum vulgare* L. x *Hordeum bulbosum* L. crosses. Genome **40**: 195-200.

Pickering, R. A., Malyshev, S., Kunzel, G., Johnston, P. A., Korzun, V., Menke, M. and Schubert, I. (2000). Locating introgressions of *Hordeum bulbosum* chromatin within the *H. vulgare* genome. Theor. Appl. Genet. **100**: 27-31.

Pickering, R. A., Steffenson, B. J., Hill, A. M. and Borovkova, I. (1998). Association of leaf rust and powdery mildew resistance in a recombinant derived from a *Hordeum vulgare* x *Hordeum bulbosum* hybrid. Plant Breed. **117**: 83-84.

Poulsen, D. M. E., Henry, R. J., Johnston, R. P., Irwin, J. A. G. and Rees, R. G. (1995). The use of bulk segregant analysis to identify a RAPD marker linked to leaf rust resistance in barley. Theor. Appl. Genet. **91**: 270-273.

Purdy, H. W., Loegering, W. Q., Konzak, C. F., Peterson, C. J. and Allen, R. E. (1968). A proposed standard method for illustrating pedigrees of small grain varieties. Crop Sci. **8**: 405-406.

Qi, X., Fufa, F., Sijtsma, D., Niks, R. E., Lindhout, P. and Stam, P. (2000). The evidence for abundance of QTLs for partial resistance to *Puccinia hordei* on the barley genome. Mol. Breed. **6**: 1-9.

Qi, X., Jiang, G., Chen, W., Niks, R. E., Stam, P. and Lindhout, P. (1999). Isolate-specific QTLs for partial resistance to *Puccinia hordei* in barley. Theor. Appl. Genet. **99**: 877-884.

Qi, X., Niks, R. E., Stam, P. and Lindhout, P. (1998). Identification of QTLs for partial resistance to leaf rust (*Puccinia hordei*) in barley. Theor. Appl. Genet. **96**: 1205-1215.

Ramsay, L., Macaulay, M., Mclean, K., Fuller, J., Edwards, K., Tuvesson, S., Morgante, M., degli Ivanissevich, S. Marmiroli, N., Maesti, E., Massari, A., Powell, W. and Waugh, R. (2000). A simple sequence repeat based linkage map of barley. Genetics **156**: 1997-2005.

Reinhold, M. and Sharp, E. L. (1982). Resistance to leaf rust of barley in Southern Texas. Cereal Rusts Bull. **10**: 4-10.

Richter, K., Schondelmaier, J. and Jung, C. (1998). Mapping quantitative trait loci affecting *Drechslera teres* resistance in barley. Theor. Appl. Genet. **97**: 1225-1234.

Roane, C. W. (1962). Inheritance of reaction to *Puccinia hordei* in barley. I. Genes for resistance among North American race differentiating varieties. Phytopathol. **52**: 1288-1295.

Roane, C. W. and Starling, T. M. (1967). Inheritance of reaction to *Puccinia hordei* in barley. II. Gene symbols for loci in differential cultivars. Phytopathol. **57**: 66-68.

Roane, C. W. and Starling, T. M. (1969). Genes conditioning reaction to *Puccinia hordei* in barley cultivars Cebada Capa and Franger. Phytopathol. **59**: 1046 (abstr.).

Roane, C. W. and Starling, T. M. (1970). Inheritance of reaction to *Puccinia hordei* in barley. III. Genes in the cultivars Cebada Capa and Franger. Phytopathol. **60**: 788-790.

Roelfs, A. P. (1988). Resistance to leaf and stem rusts in wheat. In Breeding strategies for resistance to the rusts of wheat (Simmonds, N. W. and Rajaram, S. eds.), pp. 10-22. CIMMYT, Mexico DF.

Roelfs, A. P., Singh, R. P. and Saari, E. E. (1992). 'Rust Diseasease of Wheat, Concepts and Methods of Disease Management'. (CIMMYT: Mexico).

Rodriguez, M., O'Sullivan, D., Donini, P., Papa, R., Chiapparino, E., Leigh, F. and Attene, G. (2006). Integration of retrotransposons-based markers in a linkage map of barley. Mol. Breed. **17**:173-184.

Sambrook, J., Fritsch, E. F. and Maniatis, A. (1989). Molecular cloning: a Laboratory Manual. 2nd edn. Cold Spring Harbor Laboratory Press, New York.

Schafer, J. F., Roelfs, A. P. and Bushnell, W. R. (1984). Contributions of early scientists to knowledge of cereal rusts. In The Cereal Rusts: Origin, Specificity, Structure, and Physiology (Bushnell, W. R., and Roelfs, A. P., eds.), pp. 1-32. Academic Press Inc., Sydney.

Schaper, S. (1951). Die Bedeutung der Incubationszeit fur die Zuchtung Krautfauleresistenter Kartoffel-sorten. Z. Pflzucht. **30**: 292-299.

Sharp, E. L. and Reinhold, M. (1982). Resistance gene sources of *Puccinia hordei* in barley. Plant Dis. **66**: 1012-1013.

Simkin, M. B. and Wheeler, B. E. J. (1974a). The development of *Puccinia hordei* on barley cv. Zephyr. Ann. Appl. Biol. **78**: 225-235.

Simkin, M. B. and Wheeler, B. E. J. (1974b). Overwintering of *Puccinia hordei* in England. Cereal Rusts Bull. **2**: 2-4.

Singh, D., Park, R. F. and McIntosh, R. A. (2001). Postulation of leaf (brown) rust resistance genes in 70 wheat cultivars grown in United Kingdom. Euphytica **120**: 205-218.

Singh, R. P. and Rajaram, S. (1992). Genetics of adult plant resistance to leaf rust in 'Frontana' and other three CIMMYT wheats. Genome **35**: 24-31.

Singh, S., Sindhu, J. S., Huang, N., Vikal, Y., Li, Z., Dhaliwal, H. S. and Khush, G. S. (2001). Pyramiding three bacterial blight resistance genes (xa5, xa13 and Xa21) using marker-assisted selection into indica rice cultivar PR106. Theor. Appl. Genet. **102**: 1011-1015.

Spaner, D., Shugar, L. P., Choo, T. M., Falak, I., Briggs, K. G., Legge, W. G., Falk, D. E., Ullrich, S. E., Tinker, N. A., Steffenson, B. J. and Mather, D. E. (1998). Mapping of disease resistance loci in barley on the basis of visual assessment of naturally occurring symptoms. Crop Sci. **38**: 843-850.

Stakman, E. C. and Levine, M. N. (1922). The determination of biological forms of *Puccinia graminis* on *Triticum* spp. Minn. Agric. Exp. Stn. Tech. Bull. **8**: 1-10.

Stakman, E. C. and Piemeisel, F. J. (1917). Biologic forms of *Puccinia graminis* on cereals and grasses. J. Agri. Res. (Washington DC.) **10**: 429-495.

Stakman, E. C., Stewart, D. M. and Loegering, W. Q. (1962). Identification of physiologic races of *Puccinia graminis* var. *tritici.* Agri. Res. Ser. E617. (U.S. Dept. Agri.: Washington DC).

Stam, P. (1993). Construction of integrated genetic linkage maps by means of a new computer package: JOINMAP. Plant J. **3**: 739-744.

Starling, T. M. (1956). Sources, inheritance, and linkage relationships of resistance to race 4 of leaf rust (*Puccinia hordei* Otth.) race 9 of powdery mildew (*Erysiphe graminia hordei* El. Marchal.), and certain agronomic characters in barley. Iowa State Coll. J. Sci. **30**: 438-439.

Staskawicz, B. J., Ausubel, F. M., Baker, B. J., Ellis, J. G. and Jones, J. D. G. (1995). Molecular genetics of plant disease resistance. Science **268**: 661-667.

Steffenson, B. J. and Jin, Y. (1997). A multi-allelic series at the *Rph2* locus for leaf rust resistance in barley. Cereal Rusts and Powdery Mildews Bull. **24**: 74-75.

Steffenson, B. J., Jin, Y. and Griffey, C. A. (1993). Pathotypes of *Puccinia hordei* with virulence for the barley leaf rust resistance gene *Rph7* in the United States. Plant Dis. **77**: 867-869.

Struss, D. and Plieske, J. (1998). The use of microsatellite markers for the detection of genetic diversity in barley populations. Theor. Appl. Genet. **97**: 308-315.

Stubbs, R. W. (1985). Stripe rust. In Cereal Rusts. Vol. II, Disease Distribution, Epidemiology and control. Academic Press Inc., Orlando, Florida, pp. 61-101.

Swanston, J. S. (1987). The consequences, for malting quality, of *H. laevigatum* as a source of mildew resistance in barley breeding. Ann. Appl. Biol. **110**: 351-355.

Tan, B. H. (1977a). Evaluating host differentials of *Puccinia hordei*. Cereal Rusts Bull. **5**: 17-23.

Tan, B. H. (1977b). A new gene for resistance to *Puccinia hordei* in certain Ethiopian barleys. Cereal Rusts Bull. **5**: 39-43.

Tan, B. H. (1978). Verifying the genetic relationships between three leaf rust resistance genes in barley. Euphytica **27**: 317-323.

Tautz, D. and Renz, M. (1984). Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res. **12**: 4127-4138.

Teng, P. S. and Close, R. C. (1978). Effect of temperature and uredinium density on urediniospore production, latent period, and infectious period of *Puccinia hordei* Otth. N. Z. J. Agric. Res. **21**: 287-296.

Teng, P. S. and Close, R. C. (1980). Effect of solar radiation on survival of *Puccinia hordei* uredospores in New Zealand. Cereal Rusts Bull. **8**: 23-29.

Teng, P. S., Close, R. C. and Blackie, M. J. (1979). Comparison of models for estimating yield loss caused by leaf rust (*Puccinia hordei*) on Zephyr barley in New Zealand. Phytopathol. **69**: 1239-1244.

Thomas, W. T. B., Powell, W., Waugh, R., Chalmers, K. J., Barua, U. M., Jack, P., Lea, V., Forster, B. P., Swanson, J. S., Ellis, R. P., Hanson, P. R. and Lance, R. C. M. (1995). Detection of quantitative trait loci for agronomic, yield, grain and disease charcaters in spring barley. Theor. Appl. Genet. **91**: 1037-1047.

Toojinda, T., Broers, L. H., Chen, X. M., Hayes, P. M., Kleinhofs, A., Korte, J., Kudrna, D., Leung, H., Line, R. F., Powell, W., Ramsay, L., Vivar, H. and Waugh, R. (2000). Mapping quantitative and qualitative disease resistance genes in a doubled haploid population of barley (*Hordeum vulgare*). Theor. Appl. Genet. **101**: 580-589.

Tinker, N. A. and Mather, D. E. (1995). MQTL: software for simplified composite interval mapping of QTL in multiple environments. J. Agric. Genomics, http://www.ncgr.org/ag/jag/papers95/paper295/indexp295.html.

Tranzschel, W. (1914). Kulturversuche mit uredineen in den jahren 1911-1913. Vorlauf. Mitt. Mycol. C1. **4**: 70-71.

Tuleen, I. A. and McDaniel, M. E. (1971). Location of genes *Pa* and *Pa*5. Barley News Lett. **15**: 106-107.

Udeogalanya, A. C. C. and Clifford, B. C. (1982). Control of barley brown rust, *Puccinia hordei* Otth., by benodanil and oxycarboxin in the field and the effects on yield. Crop Prot. **1**: 299-308.

Utz, H. F. and Melchinger, A. (1996). PLABQTL: A programme for composite interval mapping of QTL. J. Agric Genomics. http://www.ncgr.org/ag/jag/papers96/paper196/indexp196.html. 21/08/2004.

Van Der Biezen, E.A. and Jones, J.D.G. (1998) Plant disease-resistance proteins and the gene-for-gene concept. Trends Biochem. Sci. **12**: 454–456.

Van Der Lee, T., De Witte, I., Drenth, A., Alfonso, C. and Govers, F. (1997). AFLP linkage map of the oomycete *Phytopthora infestans*. Fungal Genet. Biol. **21**: 278-291.

Van Der Lee, T., Testa, A., van't Klooster, J., van den Berg Velthuis, G. and Govers, F. (2001). Chromosomal deletion of in isolates of *Phytopthora infestans* correlates with virulence on R3, R10, and R11 potato lines. Mol. Plant Microbe Interact. **14**: 1444-1452.

Van der Plank, J. E. (1963). Plant Diseases: Epidemics and Control. Academic Press, New York.

Van der Plank, J. E. (1968). Disease resistance in plants. Academic Press, New York/London.

Van Der Zaag, D. E. (1959). Overwinetring en epidemiologie van *Phytopthora infestans*, tevens enige nieuwe bestrijdingsmogelijkheden. Tijdschr. Plantenziekten **62**: 89-156.

Van Ooijen, J. W. and Maliepaard, C. (1996). MapQTL™ version 3.0: Software for the calculation of QTL positions in genetic maps. Wagningen, CPRO-DLO.

Varshney, R. K., Grosse, I., Hahnel, U., Siefken, R., Prasad, M., Stein, N., Langridge, P., Altschmied, L. and Graner, A. (2006). Genetic mapping and BAC assignment of EST-derived SSR markers shows non-uniform distribution of genes in the barley genome Theor. Appl. Genet. **113**: 239-250.

Varshney, R. K., Prasad, M. and Graner, A. (2004). Molecular marker maps of barley: a resources for intra- and interspecific genomics. In Biotechnology in Agriculture and Forestry. Vol. 55. Molecular Marker Systems in Plant Breeding and Crop Improvement (Lorz, H. and Wenzel, G. eds.), pp. 229-244. Springer-Verlag, Berlin, Heidelberg.

Vleeshouwers, V. G. A. A., Dooijeweert, W. A., Govers, F., Kamoun, S. and Colon, L. T. (2000). The hypersensitive response is associated with host and nonhost resistance to *Phytopthora infestans*. Planta **210**: 853-864.

Von Bothmer, R., Jacobsen, N., Baden, C., Jørgensen, R. B. and Linde-Laursen, I. (1995). An ecogeographical study of the genus *Hordeum*. 2nd eds. Systematic and Ecogeographic Studies on Crop genepools. 7. International Plant Genetic Resources Institute, Rome.

Von Wettstein-Knowles, P. (1991). Cloned and mapped genes: Current status. In Barley: Genetics, Biochemistry, Molecular Biology and Biotechnology (Shewry, P. R. ed.), pp. 73-98. CAB International, Oxford.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van De Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M. and Zabeau, M. (1995). AFLP; a new technique for DNA fingerprinting. Nucleic Acids Res. **23**: 4407-4414.

Wallwork, H., Preece, P. and Cotterill, P. J. (1992). *Puccinia hordei* on barley and *Ornithogalum umbellatum* in South Australia. Australasian Plant Pathol. **21**: 95-97.

Walther, U. (1987). Inheritance of resistance to *Puccinia hordei* Otth in the spring barley variety Trumpf. Cereal Rusts Powdery Mildews Bull. **15**: 20-26.

Walther, U. and Lehmann, C. O. (1980). Resistenzeigenschaften im Gersten-und Weizensortiment Gatersleben. 24. Prüfung von Sommer und Wintergersten auf ihr Verhalten geganuber Zwergrost (Puccinia hordei Otth.). Kulterpfanze **28**: 227-238.

Waterhouse, W. L. (1927). Studies in the inheritance of resistance to leaf rust *Puccinia anomala* Rostr. in crosses of barley. I. Proc. R. Soc. N. S. W. **61**: 218-247.

Waterhouse, W. L. (1948). Studies in the inheritance of resistance to rust of barley. II. Proc. R. Soc. N. S. W. **81**: 198-205.

Waterhouse, W. L. (1952). Australian rust studies. IX. Physologic race determinations and surveys of cereal rusts. Proc. Linn. Soc. N. S. W. **77**: 209-258.

Watson, I. A. and Butler, F. C. (1947). Resistance to barley leaf rust (*Puccinia anomala* Rost.). Proc. Linn. Soc. N. S. W. **72**: 379-386.

Watson, I. A. and Luig, N. H. (1959). Somatic hybridization between *Puccinia graminis* var. *tritici* and *Puccinia graminis* var. *secalis*. Proc. Linn. Soc. N.S.W. **84**: 207-208.

Weerasena, J. S., Steffenson, B. J. and Falk, A. B. (2004). Conversion of an amplified fragment legth polymorphism marker into a co-dominant marker in mapping of the *Rph15* gene conferring resistance to barley leaf rust, Puccinia hordei Otth. Theor. Appl. Genet. **108**: 712-719.

Wellings, C. R. (2007). *Puccinia striiformis* in Australia: a review of the incursion, evolution, and adaptation of stripe rust in the period1979-2006. Aust. J. Agril. Res. **58**: 567-575.

Wellings, C. R., Bariana, H. and Park, R. F. (2004). Revised variety response to stripe rust for Eastern Australian wheats. Cereal Rust Rep. Plant Breed. Inst. University of Sydney. **1(5)**: 1.
http://www.agric.usyd.edu.au:8888/pbi/cereal_rust_reports_crrV1_2.htm.
09/06/2006.

Wellings, C. R., Burdon, J. J., McIntosh, R. A., Wallwork, H., Raman, H. and Murray, G. M. (2000a). A new variant of *Puccinia striiformis* causing stripe rust on barley and wild *Hordeum* species in Australia. Plant Pathol. **49**: 803.

Wellings, C. R., Read, B. and Moody, D. (2000b). Stripe rust affecting barley in Australia – current and potential threats. In Procc. 8th International Barley Genetics Symposium. Adelaide, Australia, September 2000. Vol. II. pp. 197-199.

Wells, S. A. (1962). Effect of the *v* locus on yield of adapted barley varieties. Can. J. Plant Sci. **42**: 169-172.

Wenzel, P., Li, H., Carling, J., Zhou, M., Raman, H., Paul, E., Hearnden, P., Maier, C., Xia, L., Caig, V., Ovesna, J., Cakir, M., Poulsen, D., Wang, J., Raman, R., Smith, K. P., Muehlbauer, G. J., Chalmers, K. J., Kleinhofs, A., Huttner, A. and Kilian, A. (2006). A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and agricultural traits. BMC Genomics **7**: 706-727.

Wenzel, P., Raman, H., Wang, J., Zhou, M., Huttner, E. and Kilian, A. (2007). A DArT platform for quantitative bulked segregant analysis. BMC Genomics **8**: 196-206.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18**: 6531-6535.

Williams, K. J. (2003). The molecular genetics of disease resistance in barley. Aust. J. Agric. Res. **54**: 1065-1079.

Wilten, W. (1953). Het bestrijden van dwergroest (*Puccinia simplex*) in zomergerst. Zeventiende jaarb. Natl. Com. Brouwgerst. **17**: 72-79.

Wright, A. C. and Gaunt, R. E. (1992). Disease-yield relationship in barley. I. Yield, dry matter accumulation and yield-loss models. Plant Pathol. **41**: 688-698.

Yahyaoui, A., Scharen, A. L. and Sharp, E. L. (1988). Changes in virulence spectrum of *Puccinia hordei* in Tunisia. Rachis **7**: 25-31.

Yahyaoui, A. H. and Sharp, E. L. (1987). Virulence spectrum of *Puccinia hordei* in North Africa and the Middle East. Plant Dis. **71**: 597-598.

Yahyaoui, A. H., Sharp, E. L. and Reinhold, M. (1988). New sources of resistance to *Puccinia hordei* in barley land race cultivars. Phytopathol. **78**: 905-908.

Ye, G., Moody, D., Emebiri, L. and Ginkel, V. M. (2007). Designing an optimal marker-based pedigree selection strategy for parent building in barley in the presence of repulsion linkage, using computer simulation. Aus. J. Agric. Res. **58**: 243-251.

Zhong, S. B., Effertz, R. J., Jin, Y., Franckowiak, J. D. and Steffenson, B. J. (2003). Molecular mapping of the leaf rust resistance gene *Rph6* in barley and its linkage relationships with *Rph5* and *Rph7*. Phytopathol. **93**: 604-609.

Zloten, R. R. (1952). Inheritance of reaction of leaf rust in barley. M.Sc. Thesis, University of Manitoba.

Zwer, P. K., Park, R. F. and McIntosh, R. A. (1992). Wheat stem rust in Australia-1969-1985. Aust. J. Agric. Res. **43**: 399-431.

Appendices

Chapter IV:

Appendix 4.1. The distribution of BC1F2 rows and their frequencies based on the combined data of adult plant responses to *Puccinia hordei* and spike character.

Cross	Observed frequencies					χ^2 1 : 1 : 1 : 1
	A	B	C	D	Total	
Athos/2*Gus	27	15	22	17	81	4.28
Dash/2*Gus	19	13	18	15	65	1.40
Gilbert/2*Gus	19	16	13	12	60	2.00
Nagrad/2*Gus	49	30	36	35	150	5.25
Patty/2*Gus	23	23	25	18	89	1.20
Pompadour/2*Gus	37	31	34	25	127	2.48
RAH1995/2*Gus	31	23	24	18	101	3.58
Vada/2*Gus	46	44	40	36	166	1.42
WI3407/2*Gus	38	36	45	43	162	1.31
Ulandra (NT) /2*Gus	30	16	14	22	82	7.56

$\chi 2$ (3 d.f.) is 7.82 at P = 0.05

Plants from the rows included in A – Segregating for resistance and spike, B – Segregating for resistance but six row, C – Non-segregating susceptible but segregating for spike, D – Non-segregating susceptible with six row.

Appendix 4.2. Estimation of maximum recombination between the APR gene in 'Vada' and the APR gene in 'Patty', 'Pompadour', 'Nagrad', 'RAH1995', 'Athos' and 'Gilbert'.

$$2r - r^2 = 1 - \sqrt[n]{P}$$

Where n is the number of individuals and P is the specified probability

On substitution of P = 0.05

$2r - r^2 = c$ (c = 0.983, 0.987, 0.986, 0.982, 0.983 and 0.978 for 'Patty', 'Pompadour', 'Nagrad', 'RAH1995', 'Athos' and 'Gilbert', respectively)

Or

$2r - r^2 + c = 0$

By substituting into the formula for solving quadratic equations:

$$r = \frac{-b \pm \sqrt{b2 - 4ac}}{2}$$, where $ar^2 + br + c = 0$

r = 2 ± d/2 (d = 0.260, 0.228, 0.237, 0.268, 0.261, 0.297 for ‘Patty’, ‘Pompadour’, ‘Nagrad’, ‘RAH1995’, ‘Athos’ and ‘Gilbert’, respectively)

Therefore,

r = 1.13, 1.114, 1.118, 1.134, 1.130, 1.149 or 1.87, 0.886, 0.881, 0.866, 0.869, 0.851 for ‘Patty’, ‘Pompadour’, ‘Nagrad’, ‘RAH1995’, ‘Athos’ and ‘Gilbert’, respectively). Since recombination (r) cannot be greater than 0.5, there was no recombination between APR gene from ‘Vada’ and ‘Patty’, ‘Pompadour’, ‘Nagrad’, ‘RAH1995’, ‘Athos’ and ‘Gilbert’.

Chapter V:

Appendix 5.1. Frequencies of resistant and susceptible plants in segregating F3 lines from ‘Baudin’/‘PI 584760’ when inoculated with *Puccinia hordei pt.* 5453P-.

Line No.	Observed frequencies			$\chi^2_{3:1}$
	Resistant	Susceptible	Total	
4377	27	5	32	1.5
4379	23	8	31	0.01
4382	20	14	34	4.75*
4383	24	11	35	0.77
4385	22	7	29	0.01
4387	24	16	40	4.80*
4388	21	15	36	5.33*
4389	22	8	30	0.04
4390	24	5	29	0.93
4391	26	5	31	1.30
4392	20	11	31	1.82
4393	21	5	26	0.46
4394	22	4	26	1.28
4395	19	12	31	3.11
4398	21	14	35	4.20*
4399	17	8	25	0.65
4400	24	6	30	0.40
4401	21	8	29	0.10
4402	22	9	31	0.27
4404	16	13	29	6.08*
4405	22	8	30	0.04
4408	23	7	30	0.04
4409	21	9	30	0.40
4410	24	11	35	0.77
4412	18	7	25	0.12
4413	20	4	24	0.89
4417	14	7	21	0.79
4418	17	6	23	0.01
4419	23	11	34	0.98

4420	18	7	25	0.12
4422	19	10	29	1.39
4423	22	8	30	0.04
4424	24	8	32	0.00
4426	18	6	24	0.00
4427	20	10	30	1.11
4428	20	14	34	4.75*
4429	24	12	36	1.33
4432	18	10	28	1.71
4433	15	6	21	0.14
4435	16	10	26	2.51
4440	18	8	26	0.46
4441	22	7	29	0.01
4444	24	5	29	0.93
4445	19	10	29	1.39
4446	18	8	26	0.46
4447	22	6	28	0.19
4448	26	5	31	1.30
4449	17	11	28	3.05
4451	20	9	29	0.56
4455	18	8	26	0.46
4458	21	8	29	0.10
4459	24	6	30	0.40
4468	15	9	24	2.00
4473	20	8	28	0.19
4474	16	11	27	3.57
4476	19	11	30	2.18
4477	16	10	26	2.51
4480	21	10	31	0.87
4481	17	8	25	0.65
4482	19	6	25	0.01
4486	22	7	29	0.01
4490	18	7	25	0.12
4491	19	9	28	0.76
4493	17	12	29	4.15*
4496	24	6	30	0.40
4497	25	5	30	1.11
4499	20	10	30	1.11
4500	19	7	26	0.05
4501	18	8	26	0.46
4502	15	10	25	3.00
4505	18	12	30	3.60
4506	16	14	30	7.51**
4507	21	9	30	0.40
4509	15	12	27	5.44*
4511	17	13	30	5.38*
4515	23	4	27	1.50
4518	21	9	30	0.40
4522	20	13	33	3.65
4524	22	8	30	0.04

4525	18	11	29	2.59
4528	19	9	28	0.76
Total	**1631**	**714**	**2345**	**66.29**

$\chi 2$ (1 d.f.) is 3.84 and 6.64, respectively at P = 0.05 and P = 0.01
$\chi 2$ 3 : 1 (1631 : 714) = 37.12, Heterogeneity χ^2 = 29.70 (P > 0.05 at 80 d.f.)

Appendix 5.2. Frequencies of resistant and susceptible plants in segregating F3 lines from 'Ricardo'/'PI 584760' when inoculated with *Puccinia hordei pt.* 5453P-.

Line No.	Observed frequencies			χ^2 3:1
	Resistant	Susceptible	Total	
1	26	13	39	1.44
5	24	8	32	0.00
6	22	11	33	1.22
7	17	14	31	6.72**
9	19	10	29	1.39
10	21	9	30	0.40
13	19	9	28	0.76
14	21	8	29	0.10
15	21	7	28	0.00
16	24	10	34	0.35
21	23	12	35	1.61
24	22	8	30	0.04
25	26	10	36	0.15
27	23	9	32	0.17
30	19	7	26	0.05
31	16	6	22	0.06
33	21	6	27	0.11
37	17	10	27	2.09
40	20	5	25	0.33
41	17	12	29	4.15*
42	22	9	31	0.27
45	16	12	28	4.76*
50	20	8	28	0.19
52	21	11	32	1.50
53	23	10	33	0.50
57	24	9	33	0.09
61	18	9	27	1.00
66	18	14	32	3.60
67	17	10	27	2.09
68	16	9	25	1.61
70	18	13	31	3.67
74	23	6	29	0.29
76	16	8	24	0.89
84	17	7	24	0.22
85	20	11	31	1.82
86	21	9	30	0.40
87	23	11	34	0.98
89	22	8	30	0.04

91	19	8	27	0.31
92	21	6	27	0.11
93	18	5	23	0.13
94	17	13	30	5.38*
95	19	4	23	0.71
98	20	11	31	1.82
100	18	10	28	1.71
102	19	10	29	1.39
105	20	10	30	1.11
Total	**944**	**435**	**1379**	**36.72**

$\chi 2$ (1 d.f.) is 3.84 and 6.64, respectively at P = 0.05 and P = 0.01
$\chi 2$ 3 : 1 (944 : 435) = 31.50, Heterogeneity χ^2 = 5.22 (P > 0.05 at 46 d. f.)