

# Phenotypic and genotypic variation of *Puccinia hordei* in South Africa

by  
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## Declaration

I, Zizipho Spelman, declare that the Master's degree research dissertation that I herewith submit for the qualification, Magister Scientiae Agriculture in Plant Pathology at the University of the Free State, is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education.



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27/07/2021

Date

## **Dedication**

Ephesians 3:20-21, Now to Him who is able to do immeasurably more than we ask or imagine, according to His power that is at work within us, to Him be glory in the church and in Christ Jesus throughout all generations, forever and ever. For it is all because of Him. Secondly, I dedicate this thesis to my late dad, Mxolisi Headson Spelman, who always taught me to try my best and to never give up.

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indicated in red, UVPPh7231 in blue and UVPPh7235, including the isolates that typed as UVPPh7235, in green.

## List of Abbreviations

<b>Abbreviations</b>	<b>Explanation</b>
AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of molecular variance
APR	Adult plant resistance
ARC-SG	Agricultural Research Council-Small Grains
ASR	All-stage resistance
BW	Bowman
ChCl <sub>3</sub>	Chloroform
CTAB	Cetyl trimethylammonium bromide
DArT	Diversity Arrays Technology
DNA	Deoxyribonucleic acid
EC	Eastern Cape
EDTA	Ethylenediaminetetraacetic acid
Fe	Iron
$F_{IS}$	Inbreeding coefficient
FS	Free State
f. sp.	<i>forma specialis</i>
$F_{ST}$	F-statistics
$g$	Gravitational force
GBS	Genotyping by sequencing
ha	Hectare
$H_E$	Expected heterozygosity
$H_O$	Observed heterozygosity
HR	Hypersensitive response
HWE	Hardy-Weinberg equilibrium
$I_A$	Index of association
IAA	Isoamylalcohol
IT(s)	Infection type(s)
K	Potassium
$K$	“True” number of sub-populations
KZN	Kwazulu-Natal
MCMC	Monte Carlo Markov Chain
MLGs	Multi-locus genotypes
MR	Moderately resistant
MS	Moderately susceptible
N	Nitrogen
NaCl	Sodium chloride
NC	Northern Cape
NGS	Next-Generation Sequencing
NILs	Near-isogenic lines
NJ	Neighbor-joining
NW	North West
P	Phosphorus
<i>Pgt</i>	<i>Puccinia graminis</i> f. sp. <i>tritici</i>
<i>Ph</i>	<i>Puccinia hordei</i>

## LIST OF ABBREVIATIONS

PR	Partial resistance
<i>Pst</i>	<i>Puccinia striiformis</i> f. sp. <i>tritici</i>
<i>Pt</i>	<i>Puccinia triticina</i>
QTL	Quantitative Trait Loci
$r^2$	Measure corrected for the number of tested loci
R	Resistance
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
<i>Rph</i>	Reaction to <i>Puccinia hordei</i>
S	Susceptible
SA	South Africa
SNP	Single Nucleotide Polymorphism
t/ha	Ton per hectare
Tris-HCL	Tris(hydroxymethyl) aminomethane hydrochloride
UFS (UV)	University of the Free State
UK	United Kingdom
USA	United States of America
WC	Western Cape

## List of SI units

%	Percentage
°C	Degrees Celsius
cm	Centimetre(s)
kV	Kilovolt
g/kg	Gram(s) per kilogram(s)
g/L	Gram(s) per litre
g a.i./L	Gram active ingredient per litre
h	Hour(s)
kPa	Kilopascal(s)
M	Molar(s)
min	Minute(s)
m <sup>2</sup>	Square meter
mg	Milligram(s)
mg/mL	Milligram(s) per millilitre(s)
mL	Millilitre(s)
mM	Millimolar(s)
ng	Nanogram(s)
s	Second(s)
μE	Microeinstein
μg/mL	Microgram(s) per millilitre(s)
μL	Microlitre(s)
μm	Micrometre(s)
μmol	Micromolar(s)
V/cm	Volts per centimetre
v/v	Volume per volume
w/v	Weight per volume

## Abstract

Barley (*Hordeum vulgare* L.) ranks second place after wheat as the most important small grain in South Africa. Leaf rust, caused by *Puccinia hordei* G. Otth. (*Ph*), is one of the most destructive diseases directly affecting grain yield and quality of barley. The last *Ph* pathogenic survey was conducted from 1994 to 1998 describing the races SAPH 3231 (renamed UVPPh3231) and SAPH 7231 (UVPPh7231). No studies on the genetic diversity of *Ph* have been carried out in South Africa. This stresses the importance of determining the phenotypic and genotypic variation of *Ph* in SA in the current study.

In this study, 77 single pustule isolates, including one isolate each of UVPPh3231, UVPPh7231 and UVPPh7235 (Ph3\_Gt2015) were pathotyped using an international set of barley differential lines comprising of 28 entries with designated *Rph*-resistance genes and the susceptible controls Gus and PI 532013. In addition, a set of Bowman introgression lines containing resistance genes *Rph1* to *Rph15*. The phenotyping results from the two sets of differential lines were mostly in accordance, with major discrepancies recorded for BW743 (*Rph2*), BW756 (*Rph6*) and BW760 (*Rph9*). Only one new *Ph* race, UVPPh7235, was identified from the field isolates with increased virulence to *Rph3*. Seedling infection types and adult plant field and greenhouse responses were recorded for barley varieties with designated adult plant resistance genes. Baronesse (*Rph20* + *Rph24*) showed unexpected seedling resistance to isolates of all three *Ph* races. Similarly, Lenka (*Rph20* + *Rph23* + *Rph24*) showed seedling resistance to UVPPh3231 and UVPPh7231. Flagship (*Rph20*) and PI 532013 showed similar field and greenhouse adult plant responses. However, the varieties Baronesse, Gus, Lenka and Yerong (*Rph2* + *Rph23*) recorded slightly more resistant adult plant responses under greenhouse conditions. Seedling infection types and adult plant field and greenhouse responses for 10 South African barley varieties were also determined. Agulhas and Cristalia produced low seedling infection types to all three *Ph* races accompanied by moderate levels of adult plant resistance. The other varieties recorded race specific seedling resistance and were moderately susceptible to susceptible in the adult plant stage to race UVPPh7235 under field conditions. Genotyping of 48 *Ph* isolates with 20 microsatellite markers revealed five closely related genetic lineages. The low gene diversity and allelic richness levels (average 0.288 and 0.287, respectively) amongst the

## ABSTRACT

isolates, indicated low genetic diversity among local *Ph* isolates. While STRUCTURE analysis revealed three sub-populations, no clear division of the isolates into the sub-populations was evident, as all 48 *Ph* isolates were admixed for all three sub-populations. Linkage disequilibrium analysis, as well as higher  $H_0$  versus  $H_E$  values, supported the hypothesis that the South African *Ph* population is clonal, consisting of a single genetic lineage, where acquisition of new virulence occurs via single-step mutations.

Keywords: Barley, *Puccinia hordei*, leaf rust, phenotypic and genotypic variation

## Chapter 1 General Introduction

### 1.1 Barley leaf rust

Cereal grains are of major importance in the developing parts of the world. In African countries cereals provide 75% of the caloric intake required for feeding an increasing population (FAOSTAT, 2019). Many plant pathogens are known to infect and cause a negative impact on cereal production. This includes rust diseases, since the causal organisms evolved with the domestication of cereals (Singh *et al.*, 2015). Barley (*Hordeum vulgare* L.) is the fourth most important cereal crop in the world after maize, rice and wheat (Park *et al.*, 2015). From the diseases occurring on barley crops, leaf rust caused by *Puccinia hordei* G. Otth. (*Ph*) is considered to have the highest epidemic potential (Park *et al.*, 2015). Before the 1970s, the disease was considered unimportant but epidemic outbreaks have increased as a result of more intensive agricultural practices (Clifford, 1985). The disease has been reported in different barley producing regions of the world such as Australia, Europe, Ethiopia, India, South Africa (SA) and the United States (USA). There are currently 28 designated barley leaf rust resistance genes known as Response to Ph (*Rph*) genes (Park *et al.*, 2015; Kavanagh *et al.*, 2017; Ziems *et al.*, 2017; Martin *et al.*, 2020; Mehnaz *et al.*, 2021). These include *Rph20*, *Rph23* and *Rph24*, which provide adult plant resistance (APR) and *Rph1* to *Rph19*, *Rph21*, *Rph22*, *Rph25*, *Rph26*, *Rph27* and *Rph28* that are known as seedling or all-stage resistance (ASR) genes. The number of effective *Rph* genes available to breeders has decreased over the years through the appearance of *Ph* races with increased virulence. Therefore, plant pathologists and barley breeders must work continuously to characterise current resistance sources and discover novel *Rph* genes, which can be used in resistance breeding.

### 1.2 Motivation for study

In SA, barley leaf rust is chemically controlled by seed treatments and foliar fungicide applications (Van Niekerk *et al.*, 2001a; 2001b; Mr Daniel de Klerk, barley breeder ABInBev, personal communication, January 2021). There are no recent reports on the pathogenic variation of *Ph* in SA, apart from a study done 21 years ago by Van Niekerk *et al.* (2001a). Currently, breeding for resistance has become more important and is preferred over

chemical control to provide a more environmentally friendly control strategy while lowering the risk of disease outbreaks, as well as saving on input costs. However, there is no data available on the response of local barley varieties to *Ph*. This requires a study to firstly determine the race composition of *Ph* isolates collected from infected barley varieties. Following, isolates representative of the most virulent *Ph* races will then be used to determine the host responses of local barley varieties. Furthermore, it is of importance to determine effective designated ASR and APR sources to isolates of local races for *Ph*-resistance. The use of molecular markers is essential to understand and determine the genotypic variability and structure of *Ph* isolates in SA. According to Gnocato *et al.* (2018), knowledge of the genetic structure of pathogens can aid in predicting and minimising disease and assist in compiling breeding strategies. Pretorius *et al.* (2007) stated that phenotyping should be supported by genotyping. Results of rust surveys and pathotyping combined with genotyping can provide a better understanding of the evolution of the pathogen in a specific region. Results obtained from this study will aid local breeders in developing a breeding strategy to *Ph*. The outcome will further contribute to our understanding of the phenotypic and genotypic diversity of *Ph* isolates in SA.

### **1.3 Objectives of research**

#### Objective 1: Determining the dominant barley leaf rust races in South Africa and the response of key germplasm sources

Surveys and race typing studies are required to determine the *Ph* races occurring in SA and the response of non-differential germplasm sources to isolates representative of the detected races. This will include barley lines carrying 28 designated resistance genes, Bowman (BW) introgression lines and commercial varieties. Evaluations will include seedling and adult plant assessments of barley entries in greenhouse and field trials. Data generated will provide valuable information to breeders on currently available and potential new sources of genetic resistance. Barley producers will benefit from the variety response data to develop control strategies. This include variety selection and timing of fungicide application when environmental conditions are favourable for disease outbreaks.

Objective 2: Genotypic characterisation of *Puccinia hordei*

Genetic variation amongst *Ph* isolates can provide an indication of the potential of the pathogen to evolve, including the likelihood to develop resistance to fungicides, to survive during unfavourable environmental conditions or to overcome newly deployed resistance genes. This will be the first attempt to determine the genetic variability for *Ph* in SA using microsatellite markers. Microsatellite markers described by Karaoglu and Park (2014), were found informative in determining genotypic variation among *Ph* isolates and will be used to determine the genotypic variability among South African *Ph* isolates. Different statistical analyses will be explored to reflect on genetic diversity amongst local *Ph* isolates, grouping of *Ph* isolates into sub-populations, determining the correlation between genotype and phenotype of *Ph* isolates and provide an indication as to whether new races are likely to evolve through asexual or sexual reproduction.

## Chapter 2 Literature Review

### 2.1 Barley (*Hordeum vulgare*)

#### 2.1.1 Background

Cereal grains are thought to have developed from a common ancestor due to the similarities in their appearance and the pathogens affecting them (Pourkheirandish and Komatsuda, 2007). Cereal improvement is of major importance in developing countries, particularly in Africa where cereals provide between 55-70% of the required caloric intake (FAOSTAT, 2020). Numerous pathogens are known to infect cereals. An example is the rusts which have evolved since cereals have become domesticated (Singh *et al.*, 2015).

Barley (*Hordeum vulgare* L. subsp. *vulgare*) is a monocotyledonous plant belonging to the Poaceae, formerly known as the Gramineae, family (Von Bothmer and Komatsuda, 2011). The genus *Hordeum* consists of 33 species and 45 taxa, with only *H. vulgare* being domesticated (Blattner 2009; Jakob *et al.*, 2014). The haploid genome of *H. vulgare* consists of  $n=7$  (Von Bothmer and Komatsuda, 2011), while it could either be diploid ( $2n=14$ ) (*H. vulgare*), tetraploid ( $4n=28$ ), or hexaploid ( $6n=42$ ) (Wicker *et al.*, 2009). According to Park *et al.* (2015), barley ranks fourth as one of the most important cereal crops after maize, rice and wheat. Barley is a hardy crop and can be grown in poor environmental conditions, while still producing a reasonable yield compared to other cereal crops (Park *et al.*, 2015). It is therefore often referred to as a poor man's crop, due to low production costs and lower quality compared to other cereals.

Due to its adaptive nature, barley has a short growing period which enables early maturity to avoid plant stress (Park *et al.*, 2015). Compared to other cereal crops such as wheat, barley grain is low in protein, calcium and phosphorous but high in carbohydrates (Grando and Macpherson, 2005). Barley has multiple uses, such as industrial sage (manufacturing and packaging cellulose pulps), livestock feed, human food (bread), but is mostly used for beer malting (DAFF, 2019).

### 2.1.2 Barley production

In terms of global production of barley recorded for the 2019/2020 season, countries such as the European Union (including Spain, Germany and France), Russia, Australia and Canada are major contributors, while SA, Zimbabwe and Israel can be regarded as minor contributors when comparing the number of tons produced in each country (Table 2.1) (STATISTA, 2021). In SA, the main barley production provinces are the Western Cape (WC), Northern Cape (NC) and North West (NW) provinces with isolated production areas in the Free State (FS), Eastern Cape (EC) and KwaZulu-Natal (KZN) provinces (DAFF, 2019). While the WC dryland cultivation area is much larger than the irrigation areas in terms of hectares planted, the irrigation areas produce higher yields per hectare (Figure 2.1 and Figure 2.2) (SAGIS, 2021). The total South African barley production (Figure 2.3) from 2000 to 2021 emphasised the importance of the WC dryland region, as most of the barley used for beer malting is produced in this region. Figure 2.3 illustrates an increase in the production of cultivated barley in SA over the years, however with fluctuating yield which could be influenced by several factors including inconsistent rainfall and disease outbreaks.

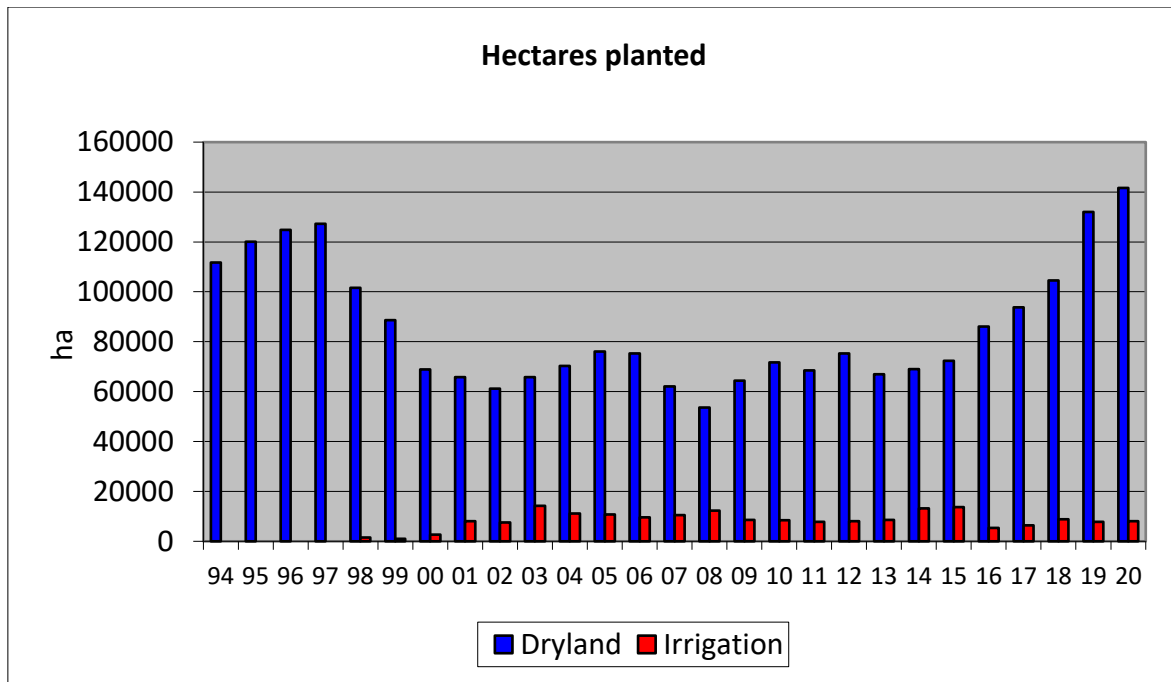
## 2.2 *Puccinia hordei* (barley leaf rust)

### 2.2.1 Background

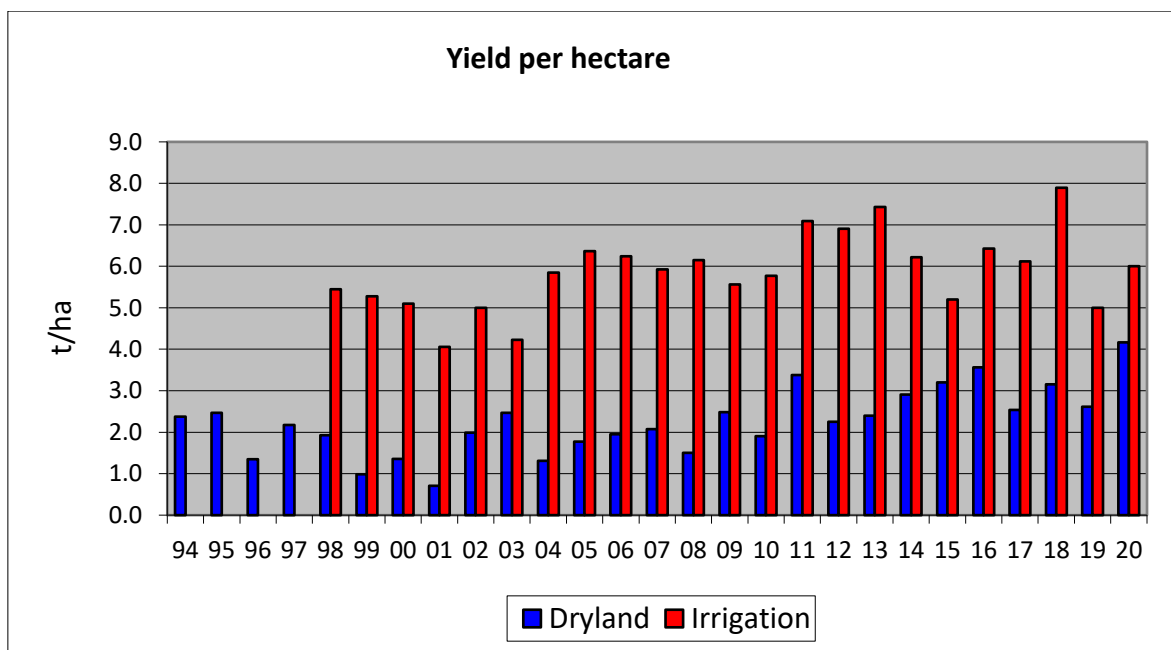
Plant pathogens pose a significant constraint on the production of barley, with disease outbreaks often resulting in crop losses and lower grain quality (Cotterill *et al.*, 1992). Rust fungi of Basidiomycota are considered one of the major causal disease agents of small grains and are obligate plant pathogens with complex life cycles (Hiratsuka and Sato, 1982; Park *et al.*, 2015). These pathogens are well studied and due to spore dispersal by wind, they pose a high potential to cause detrimental disease outbreaks and epidemics around the world (Park, 2000). There are four important rust diseases of barley. These are stripe rust (caused by *Puccinia striiformis* Westend. f. sp. *hordei* Erikss.), stem rust (caused by *Puccinia graminis* Pers.), crown rust (caused by *Puccinia coronata* Corda var. *hordei* Jin & Steff.) and the most commonly occurring leaf rust (caused by *Ph*) (Park *et al.*, 2015).

**Table 2.1** Barley producing countries in the world and their contribution during the 2019/2020 season to the world production of 157 186 000 tons. (STATISTA, 2021)

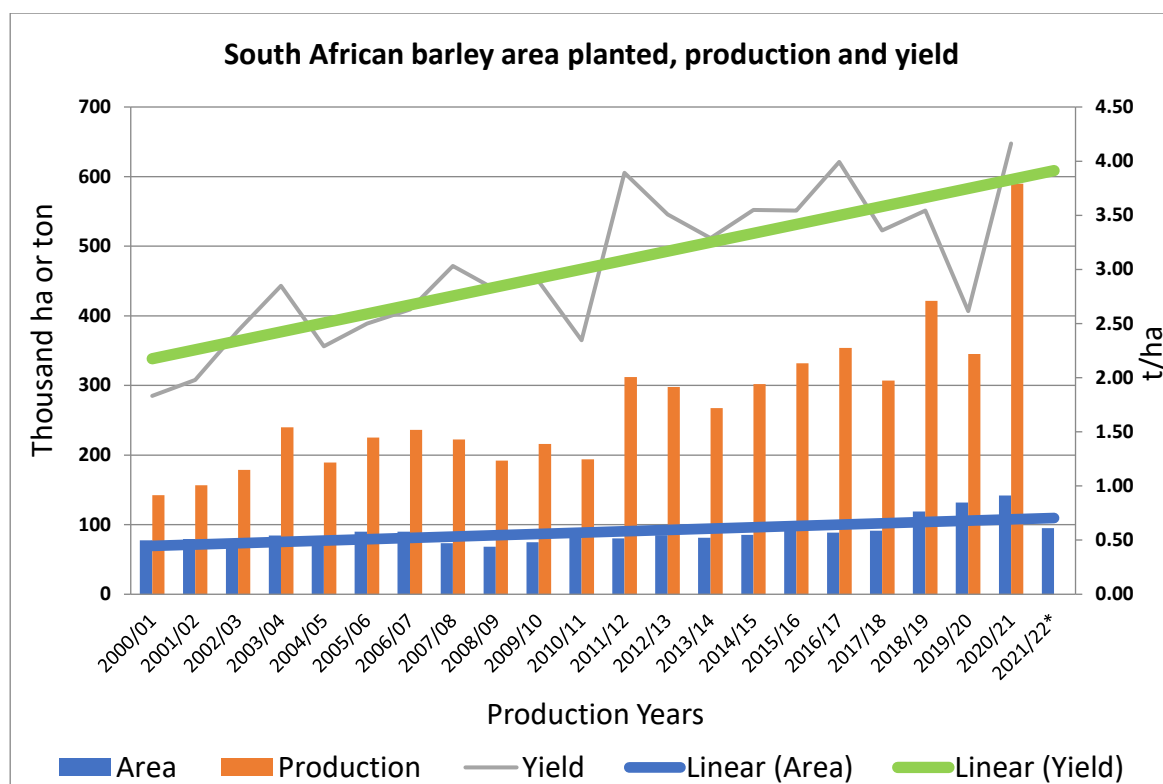
Country/region	Ton	Country	Ton
European Union	63 100 000	Chile	220 000
Russia	20 600 000	Peru	200 000
Australia	11 000 000	Switzerland	185 000
Canada	10 741 000	Uzbekistan	180 000
Turkey	8 100 000	South Korea	144 000
Ukraine	8 000 000	Moldova	135 000
Kazakhstan	3 800 000	Tajikistan	135 000
Iran	3 750 000	Egypt	108 000
Argentina	3 700 000	Libya	100 000
United States of America	3 600 000	North Macedonia	100 000
Ethiopia	2 350 000	Armenia	82 000
Syria	1 900 000	Kenya	80 000
Algeria	1 845 000	Bosnia	72 000
India	1 687 000	Eritrea	65 000
Iraq	1 550 000	Pakistan	63 000
Belarus	1 050 000	Zimbabwe	55 000
Mexico	1 000 000	Bolivia	47 000
Azerbaijan	970 000	Georgia	46 000
China	900 000	Nepal	31 000
Uruguay	670 000	Lebanon	31 000
Tunisia	664 000	Turkmenistan	23 000
Morocco	640 000	Yemen	21 000
Norway	600 000	Jordan	20 000
Serbia	500 000	Colombia	20 000
South Africa	500 000	Ecuador	12 000
Kyrgyzstan	500 000	Saudi Arabia	10 000
Brazil	385 000	Bangladesh	7 000
New Zealand	385 000	Albania	7 000
Japan	250 000	Bhutan	5 000
Afghanistan	240 000	Israel	5 000



**Figure 2.1** Comparing the total hectares planted with barley under dryland conditions and irrigation in South Africa for the seasons 1994 to 2020 (SAGIS, 2021).



**Figure 2.2** Mean barley yield in ton/ha achieved from 1994 until 2020 under dryland conditions and irrigation in South Africa (SAGIS, 2021).



**Figure 2.3** South African barley area planted, production and yield achieved from production year 2000 to 2021 (GrainSA, 2021).

Leaf rust is also referred to as brown rust of barley and is found in most barley production areas in the world (Clifford, 1985). In SA, the disease is commonly found in the WC province (Van Niekerk *et al.*, 2001a). However, there are no reports of this disease occurring on barley planted under irrigation in the summer rainfall areas. Leaf rust of barley was first recorded in the WC province in 1953 (Van Niekerk *et al.*, 2001a). The common occurrence and regular impact of barley leaf rust have triggered a need for disease control research. Various integrated methods can be applied to control *Ph*, including chemical control, breeding for resistance and cultural practices.

### 2.2.2 Taxonomy

The family Pucciniaceae contains the most important fungal pathogens after *Magnaporthe oryzae* (rice blast) and *Botrytis cinerea* (grey mould) (Dean *et al.*, 2012). This family includes 4000 to 7000 species described under the genus *Puccinia* (Clifford, 1985; Singh *et al.*, 2015). The classification of *Ph* is summarised in Table 2.2 (Clifford, 1985; Toome and Aime, 2012). According to Stevenson and Johnson (1946), the barley leaf rust pathogen was first

described as *Uromyces hordei* by Nielsen and in 1875, as *P. anomala* Rostr. In 1894 it was re-named *Puccinia simplex* Erikss. and E. Henn, but finally accepted as *Ph* in 1871 (Park *et al.*, 2015). This led to differences in the use of common disease names including, brown and dwarf rust of barley.

**Table 2.2** Botanical classification of *Puccinia hordei*.

Taxon	Eukaryota
Kingdom	Fungi
Phylum	Basidiomycota
Order	Pucciniales
Family	Pucciniaceae
Class	Pucciniomycetes
Genus	<i>Puccinia</i>
Species	<i>Hordei</i> Otth
Scientific name	<i>Puccinia hordei</i> Otth

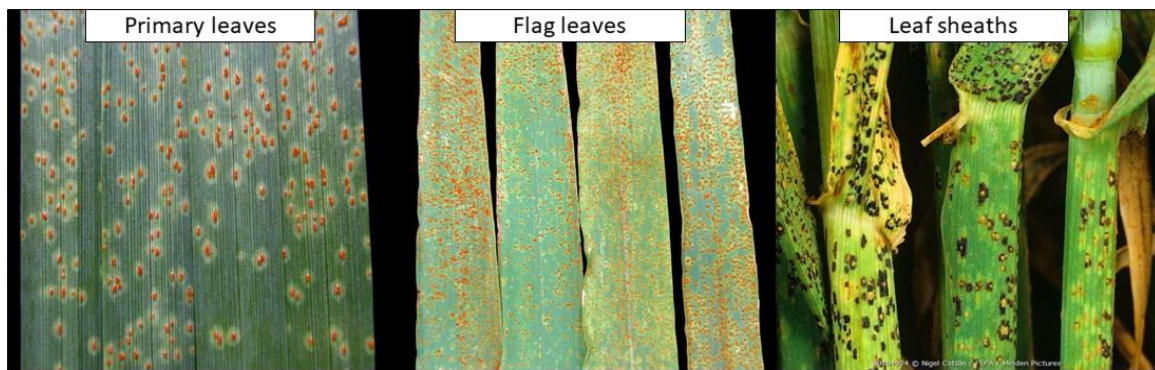
### 2.2.3 Biology and life cycle

Infection of barley with *Ph* is first visible as small chlorotic flecks which later develop into uredinia containing urediniospores (Roane, 1972). When susceptible to *Ph*, the barley leaf blades and sheaths produce orange to brown uredinia (Figure 2.4). Scattered uredinia are often surrounded by chlorotic halos which can occur on both sides of the leaf blade or on the outside of the leaf sheath. Severe infections can lead to the entire leaf becoming infected. Late infection is often associated with necrosis and chlorosis of plant tissue followed by the production of dark (black) telia containing teliospores (Figure 2.4). Scholes and Farrar (1987) reported that fungal mycelia within the leaves are directly proportional to the increase of individual pustules. Murray *et al.* (1998) stated that after the establishment of the pathogen, transpiration and respiration within the plant increase while the opposite happens to photosynthesis. Infection can severely impact plant growth which may result in the stunted appearance of the plants and yield loss, due to poor seed and grain quality (Melville and Lanham, 1972).

*Puccinia hordei* is a highly specialised biotrophic pathogen that can infect about 20 plant species (Clifford, 1985) and is regarded as an obligate parasite. Furthermore, *Ph* is a macrocyclic, heteroecious rust pathogen that produces five different spore types and requires more than one host plant to complete sexual recombination (Clifford, 1985). The

pathogen is heterothallic and needs different mating types to fuse prior to sexual recombination, thus requiring both *Hordeum* (the host) and *Ornithogalum* (the alternate host) to complete its full life cycle (Clifford, 1985) On *Hordeum* spp. (primary host), uredinia and telia are produced, whereas on *Ornithogalum*, *Leopolia* and *Dipcadi* spp. (secondary or alternate hosts) of the Liliaceae family, aecia and pycnia are produced (Anikster and Wahl, 1979).

Tranzschel (1914) identified the genus *Ornithogalum* as the alternate host of *Ph*. Later, Anikster (1982) successfully infected different *Ornithogalum* spp. with *Ph* basidiospores from different *Hordeum* spp. such as *H. vulgare*, *H. spontaneum*, *H. bulbosum* and *H. murinum*. Pycnial fertilisation was confirmed in all the alternate host species. In Europe and Egypt, the season and growth of *Ornithogalum* and the production of teliospores do not coincide and the sexual cycle is therefore thought to be unimportant (Clifford, 1985).

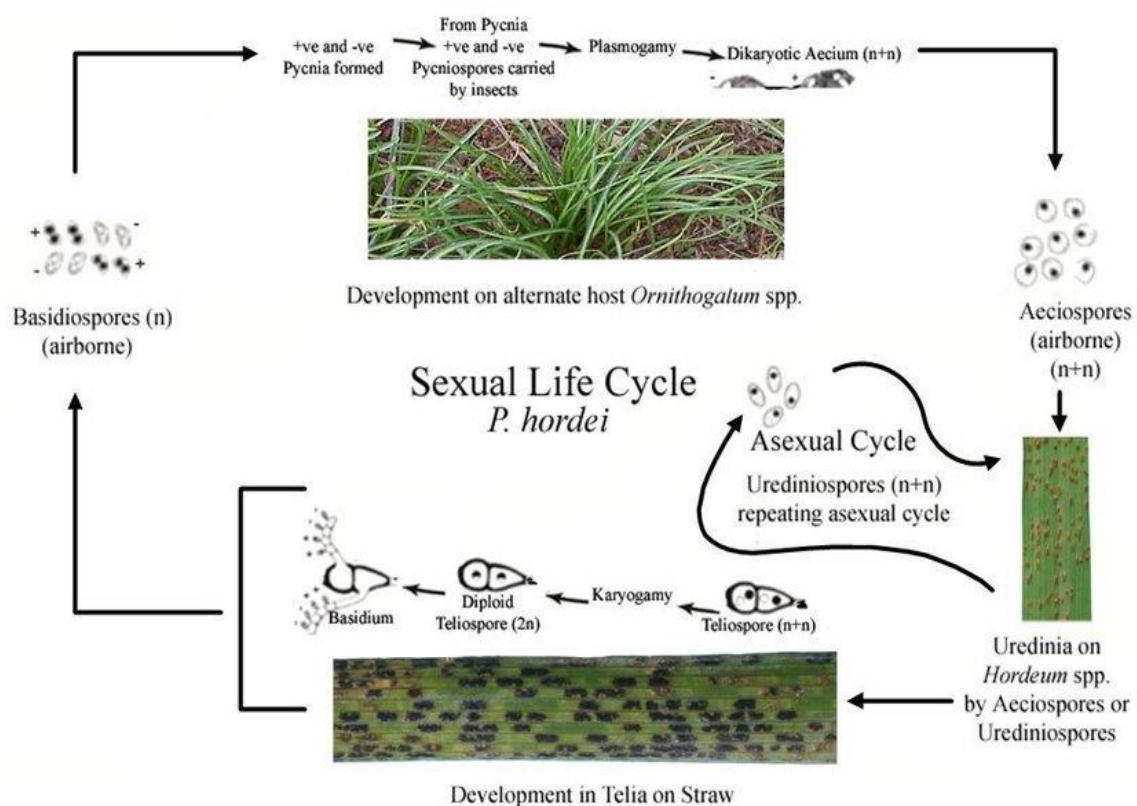


**Figure 2.4** The appearance of *Puccinia hordei* uredinia and telia on the leaves and leaf sheaths of barley. From left to right: uredinia producing urediniospores on primary and flag leaves of a susceptible barley variety 14 days after inoculation (Photo, WHP Boshoff); telia producing teliospores on leaf sheaths of barley stems (Photo, Nigel Minden pictures; <https://www.mindenpictures.com>).

In South Australia, the alternate host *O. umbellatum* (Star of Bethlehem) is a common weed (Wallwork *et al.*, 1992), thus allowing the completion of the pathogen's life cycle (Park, 2003). In the WC barley producing region, about 54 species of *Ornithogalum* coexist with *Hordeum* spp. (Van Niekerk *et al.*, 2001a). However, the sexual cycle of *Ph* has never been reported in SA. Thus, although several *Ornithogalum* spp. are indigenous to the WC province and their growth coincides with commercial barley plantings and *H. murinum* L.

accessions that are susceptible to *Ph* infection, aecial infections have not been confirmed. This makes sexual recombination unlikely in the WC barley production region and imply that *Ph* over-summer in SA through infection of volunteer barley or *H. murinum* accessions and that urediniospores act as primary inoculum (Van Niekerk *et al.*, 2001a).

At the end of the reproductive growth of mature barley plants, dark thick-walled telia with dikaryotic teliospores are produced on the leaves and stems (Figure 2.5). Teliospores are produced through karyogamy involving the fusion of two haploid nuclei. These teliospores may germinate or stay dormant through a resting spore stage. During dormancy, teliospores remain intact inside telia in infected stubble through winter. When spring starts, teliospores germinate to form a basidium from each cell through meiosis which produces four unicellular haploid (n) basidiospores. Basidiospores have no impact on the *Hordeum* spp., but can infect alternate hosts if present. As in the case of Australia where alternate hosts are present, basidiospores germinate and produce a haploid mycelium which take over the leaf tissue (Clifford, 1985).



**Figure 2.5.** Diagrammatic representation of the asexual and sexual infection cycles of *Puccinia hordei* by Dr. Karanjeet Sandhu (Sandhu, 2011).

Pycnia is characterised by a fruiting body forming on both sides of the leaf with an oogonium developing through an encircled antheridium that is scattered and spherical to ellipsoid in shape. Pycnia are 86-144 x 99-158  $\mu\text{m}$  in diameter with a black-yellow colour (Clifford, 1985). Pycniospores are pear-shaped and 2 x 3-5  $\mu\text{m}$  in diameter and are produced in a sticky honeydew that is attractive to insects. Insects carry pycniospores between pycnia as they feed on the honeydew resulting in cross-fertilisation (Anikster *et al.*, 1999). Fertilisation produces a dikaryotic aecium forming aeciospores through plasmogamy, which is known as the fusion of cytoplasm without the fusion of the nuclei. Fertilisation of genetically different female and male gametes of pycniospores will result in aeciospores with potentially new virulence combinations. Aeciospores are dispersed by wind, leading to the infection of *Hordeum* spp., later producing a dikaryotic mycelium from which uredinia is produced, thereby initiating the beginning of a new disease cycle (Clifford, 1985).

Aecia have a cup-shaped appearance and are yellow with a diameter of 200-300  $\mu\text{m}$  (Clifford, 1985). The outer envelope of the sporophore cells is transparent and composed of a 3-4  $\mu\text{m}$  thick coarsely warty internal wall and 6-8  $\mu\text{m}$  thick smooth external wall (Clifford, 1985). Aeciospores are sub-transparent, thickly warty and up to 18-26 x 16-33  $\mu\text{m}$  in size with a globoid shape (Clifford, 1985). Uredinia is similar to pycnia and aecia in terms of the position of the fruiting bodies but differ by their orange-brown colour. Urediniospores are subgloboid, yellow in colour and range between 21-34 x 15-24  $\mu\text{m}$  in diameter (Clifford, 1985). Telia is blackish-brown in colour with a 15-24 x 34-56  $\mu\text{m}$  diameter and contains angular oblong teliospores that are honey-blackish in colour (Peterson, 1974; Clifford, 1985).

The initial *Ph* urediniospore or aeciospore inoculum originates from over-wintered or over-summered volunteer *Hordeum* spp. or the alternate hosts (Clifford, 1985). A common source of initial inoculum is the presence of a green bridge consisting of self-seeded volunteer barley plants anywhere around the barley field that can act as resource of urediniospores for the next infection cycle (Clifford, 1972; Clifford, 1985). Absorption of low molecular weight ionic material begins once the urediniospore is on the leaf and exposed to dew at night. This leads to the hydration of the spores (Simkin and Wheeler,

1974). Policy and Clarkson (1978) reported that at an optimum temperature of 5-25°C during hydration, a germ tube will be produced. Once germinated, a dome-shaped structure called an appressorium is formed, which later penetrates through the stomatal guard cells with a penetration peg (Clifford, 1985). Intense penetration will occur at temperatures of 10-20°C (Simkin and Wheeler, 1974). Clifford (1972) explained that bilateral infection hyphae are produced from a sub-stomatal vesicle that forms mycelia with haustoria, which is used for nutrition absorption without killing the host cells.

Sporulation begins 6-8 days after infection (Policy and Clarkson, 1978) and the process may be prolonged up to 60 days if temperatures as low as 5°C prevail (Simkin and Wheeler, 1974). Uredinia rupture through the epidermis are visible as orange-brown rust pustules (Figure 2.5), which results in the release of urediniospores. Urediniospores are dispersed by wind and can remain viable during cloudy conditions for a period of 38 days, while sunny weather will rapidly reduce viability (Teng and Close, 1980).

#### **2.2.4 Pathogenic variation**

Stakman and Piemeisel (1917) were the first to identify rust isolates that were phenotypically different. They were studying wheat stem rust caused by *P. graminis* Pers. f. sp *tritici* Erikss. & Henning (*Pgt*). There are different ways in which pathogenic variation can result. Sexual recombination requires completion of the sexual life cycle leading to greater genetic diversity as opposed to asexual reproduction (Morrow and Fraser, 2009). Exotic introductions of pathotypes, mutations and somatic hybridisation have been sources of variation in the absence of sexual recombination (Groth and Roelfs, 1982; Drake *et al.*, 1998; Park *et al.*, 1999; Park 2003). New pathotypes or races result from these processes have been reported in different parts of the world. *Puccinia hordei* is the only cereal rust pathogen for which sexual reproduction has been reported in Australia (Wallwork *et al.*, 1992; Park, 2008). Besides Australia, sexual reproduction of *Ph* has been reported to occur in Greece (Critopoulos, 1956) and Israel (Manisterski, 1989).

The introduction of exotic incursions of rust pathogens due to the dispersal of urediniospores from one production area to another, may further result in pathogenic variation. Visser *et al.* (2012) postulated that through urediniospore dispersal, races of the

wheat stem rust pathogen have spread from Zambia and Zimbabwe to SA. In a further study, Visser *et al.* (2019) reported that shared genetic similarity between South African and Australian races of *Pgt* can be explained through the movement of urediniospores from Africa to Australia.

Single-step mutations can result in pathogenic variation and are considered most common in the development of new pathotypes of cereal rust diseases (Park *et al.*, 2015). Intense selection pressure of a rust pathogen occurs through the cultivation of the same resistant variety over large areas or different varieties with the same resistance gene, this often results in the appearance of new pathotypes with the required virulence. This is commonly referred to as the “boom-and-bust cycle” (Hulbert and Pumphrey, 2014). Van Niekerk *et al.* (2001a) reported that the extensive planting of the leaf rust susceptible barley variety Clipper explained the lack of pathotype variation for *Ph* in SA. However, the shift to the leaf rust resistant variety SSG 532 which contained the *Rph12* resistance gene, resulted in the appearance of a new pathotype rendering this gene ineffective. With no proof of sexual recombination occurring in SA, this shift in virulence was attributed to a single-step mutation in *Ph*. The same occurred in Australia as large-scale cultivation of the varieties Franklin, Lindwall, Fitzgerald and Tallon reportedly resulted in the appearance of several new *Ph* pathotypes including 4610P<sup>+</sup>, 5435P<sup>-</sup> and 5610P<sup>+</sup> gaining virulence to *Rph12* (Park, 2003; Park, 2008).

Lastly, another process that generate pathogenic variation is somatic hybridisation. To date, no reports exist indicating the occurrence of somatic hybridisation in *Ph*. However, evidence of this mechanism was reported for *Puccinia triticina* Erikss. (*Pt*) in Australia (Park *et al.*, 1999). Another example involves scabrum rust, which reportedly resulted from the fusion of *P. graminis* Pers. f. sp. *secalis* Erikss. & Henning and *Pgt* when both pathogens infected barley (Dracatos *et al.*, 2015).

### **2.3 Economic importance**

According to Clifford (1985), barley leaf rust caused by *Ph* was initially considered as economically unimportant. The economic importance of the disease increased significantly with more frequent epidemics reported in barley producing countries (Van Niekerk *et al.*,

2001b). The occurrence of the disease varies over seasons and across countries which can be attributed to the adoption of more intensive cultivation practices and an increase in the demand for barley production (Park *et al.*, 2015). Leaf rust is considered the most important rust disease of barley causing regular yield losses in regions like Australia, Europe, New Zealand, Ethiopia, North Africa, SA, the Czech Republic, the United Kingdom (UK) and the USA (Park *et al.*, 2015).

From literature, there is no record of complete crop loss during outbreaks of barley leaf rust. In Canada, Newton *et al.* (1945) reported yield losses of 24% in untreated control plots with the application of sulfur dust to control leaf rust. The loss in yield was attributed mainly to reduced kernel weight. Yield losses of up to 30% were reported in Mexico on plantings of the variety Esmeralda which included reduced malting value of the harvested grain (Gonzales *et al.*, 2013). In Uruguay, yield losses of up to 25% were recorded due to *Ph* infection on barley (Pereyra and Ackermann, 2007). In Australia, up to 31% of yield losses were reported by Cotterill *et al.* (1995). The historic importance of barley leaf rust in New Zealand and Australia is emphasised when considering recorded yield losses compared to other countries. In New Zealand, 21% yield losses were estimated during outbreaks of the disease, due to lower grain weight (Arnst *et al.*, 1979). Early infections were more damaging with yield losses of 32% recorded compared to late infections with 10% losses. Late occurring co-infections of *Ph* and powdery mildew were most damaging with yield losses as high as 52% (Lim and Gaunt, 1986). In Australia, yield losses as high as 62% were recorded on susceptible varieties (Cotterill *et al.*, 1992).

In SA, there has been an increase in the demand for barley grains of consistent and high malting quality. In fungicide field trials conducted by Van Niekerk *et al.* (2001b), six barley varieties namely Clipper, Stirling, SSG 525, SSG 532, B49/2 and B95/10 were included. Results showed that Clipper, Stirling and SSG 525 were highly susceptible whereas B49/2, B95/10 and SSG 532 were considered resistant to *Ph*. Yield losses of 58%, 49% and 39% were recorded for the highly susceptible varieties and 5%, 20% and 13% for the resistant varieties, respectively. Regression analysis confirmed a strong positive correlation ( $R^2 = 0.7216$ ) between yield loss and disease severity. The South African grading is strict for characteristics like kernel plumpness and percentage siftings, which, except for variety

B94/2, were significantly reduced by *Ph* infection in the other five varieties. Barley leaf rust severity further influenced the date of maturity and lodging, thus indirectly impacting other parameters as well (Van Niekerk *et al.*, 2001b).

## **2.4 Disease control**

Various methods can be effectively applied to control *Ph*. These methods include cultural practices, chemical control and resistance breeding. The latter is considered the most economical and sustainable method of disease control. Control strategies like clean tillage during the off-season, seed-dressing with fungicides and the application of foliar fungicides can be considered when resistant varieties are not available.

### **2.4.1 Cultural methods**

Different tillage practices are applied in barley production which is mainly aimed at conserving moisture, as well as seed-bed preparation (Walters *et al.*, 2012) to reduce the source of inoculum. However, since *Ph* is dispersed through wind, crop rotation and tillage practices are less applicable to control *Ph* compared to soil-borne pathogens. The removal of *Ph* susceptible volunteer barley plants during the off-season (summer in SA) and stubble borne diseases is likely to result in lower early-season inoculum (Van Niekerk *et al.*, 2001a). This can be done by grazing, tillage or the application of herbicides through destruction of the green-bridge (Boshoff *et al.*, 2000). *Puccinia hordei* often develop later in the season, thus implementing an earlier planting date where possible, may result in disease escape in some production areas or seasons (Paulitz and Steffenson, 2011). However, in SA the optimum planting window is only a few weeks making this management strategy irrelevant.

Knott (1989) described implementing cultural practises in rust epidemics as a way to break the life cycle of the pathogen by eradicating the alternate host. The role of sexual recombination in generating genetic variability in rust fungi is well documented. The eradication of barberry plants (*Berberis* spp.) in the USA from 1918 to 1980 on which *Pgt* undergoes sexual recombination, had a large effect on reducing genetic diversity and increasing the stability of *Pgt* races collected from wheat (Roelfs, 1982). Among many of

the susceptible *Berberis* spp. potentially serving as alternate hosts, the common barberry (*B. vulgaris*) is most cosmopolitan and has been targeted for eradication by farmers as a control measure for decades in Europe and North America (Jin, 2011). This practice contributed to the successful control of stem rust epidemics by eliminating local sources of initial inoculum in many regions where the rust cannot survive at the uredinial stage year-round due to a lack of host plants and/or unfavourable climatic conditions (Roelfs, 1982).

Eradication of barberry also led to an unintended, but highly beneficial, 'side effect', by eliminating a powerful means by which the pathogen undergoes genetic recombination (Roelfs and Growth, 1980). Disruption of the life cycle of the stem rust pathogen, in combination with the use of host resistance, has had far-reaching consequences. Stem rust populations on wheat around the world experienced a steady decline. Existing *Pgt* populations in the eastern United States consist of one or two races, which reproduce clonally. In Western Europe, stem rust has been absent for several decades. A report by Lewis *et al.* (2018) however implied the potential re-emergence of the disease in the UK. Since the alternate host of *Ph* is not known to be of importance in SA, this control strategy is of no use as the SA population is known to have developed through asexual reproduction. Furthermore, there are no reports in literature on the breaking of the life cycle of *Ph* through control of its alternate host.

#### **2.4.2 Chemical control**

Seed-dressing with an appropriate fungicide can control rust pathogens on susceptible varieties during early growth stages (Carmona *et al.*, 2020). However, without any follow-up foliar fungicide application, plants do become susceptible to infection during the tillering stage. In SA, Van Niekerk *et al.* (2001b) reported yield increases varying from 4.8% to 58.3% and low levels of infection on barley varieties when treated at three-week intervals with the triazole fungicide tebuconazole following inoculation with *Ph*. These results pointed at a positive yield effect from applying tebuconazole at consecutive intervals other than *Ph* control, as yield increases were also reported on barley varieties that showed low levels of infection (Van Niekerk *et al.*, 2001b). Another report stated that a single application of a triazole fungicide is enough to protect barley from leaf rust (Das *et*

*al.*, 2007). Chemical control may become uneconomical if more than one fungicide application is needed during the season (Gonzales *et al.*, 2013). The repeated application of fungicides may also result in selection pressure on plant pathogens to quickly evolve with the consequent development of fungicide tolerance or even resistance (Tucker *et al.*, 2013), even though rust pathogens are reportedly less likely to develop fungicide resistance (Walters *et al.*, 2012). In SA, there are no seed treatments registered for controlling *Ph*. In practice, the fungicide Redigo® with the triazole prothioconazole as an active ingredient may provide seedling protection from *Ph* infection for up to six weeks after planting (Mr Daniel de Klerk, barley breeder ABInBev, personal communication, January 2021).

Fungicides with different active ingredients are registered in SA to control *Ph* (AVCASA, 2021; Table 2.3). According to Mr Daniel de Klerk, barley producers commonly apply fungicides twice during the growing season on commercial barley plantings in the south WC province to control a complex of diseases, which includes barley leaf rust. The residual effect of these fungicides is generally between 3 to 5 weeks. Curative fungicides work within the plant tissue, thereby preventing the early onset of pathogen growth inside the plant tissues and become effective between one to three days after application, while protective fungicides work prior to disease development acting as a barrier against infection (Mueller and Robertson, 2008).

#### **2.4.3 Breeding for resistance**

Breeding for resistance is considered the most effective and sustainable disease management tactic to control leaf rust (Zillinsky, 1983). In order to be successful in resistance breeding to barley leaf rust, a thorough understanding of the genetic base of resistance sources is required (Clifford, 1985). Waterhouse (1927) conducted the first study to explain the inheritance of resistance to *Ph* in barley, paving the way for recent studies including ASR, Partial Resistance (PR) and APR. Currently, there are 28 designated *Rph*-resistance genes, namely *Rph1* to *Rph28*. These include 25 ASR (*Rph1* to *Rph19*, *Rph21*, *Rph22*, *Rph25*, *Rph26*, *Rph27* and *Rhp28*) and three APR (*Rhp20*, *Rph23* and *Rhp24*) genes originating from *H. vulgare*, *H. vulgare* ssp. *spontaneum* and *H. bulbosum*.

**Table 2.3** Fungicides, their trade names, active ingredient(s) and the gram active ingredient per litre (g a.i./L) for the control of *Puccinia hordei* on barley in South Africa. (AVCASA, 2021)

Trade name	Active ingredient(s)	g a.i./L
Obstructo	Azoxystrobin	250 g/L
Amistar Xtra	Azoxystrobin/Cyproconazole	200/80 g/L
Discovery	Azoxystrobin/Cyproconazole	200/80 g/L
Hyper 28	Azoxystrobin/Cyproconazole	200/80 g/L
Az-Well	Azoxystrobin/Epoxiconazole	125/125 g/L
Estricon	Azoxystrobin/Epoxiconazole	125/125 g/L
Improve	Azoxystrobin/Epoxiconazole	125/125 g/L
Custodia	Azoxystrobin/Tebuconazole	120/200 g/L
Duett	Carbendazim/epoxiconazole	125/125 g/L
Clout	Carbendazim/flusilazole	125/120 g/L
Collosa	Carbendazim/flusilazole	125/120 g/L
Flusilazim	Carbendazim/flusilazole	125/120 g/L
Punch C	Carbendazim/flusilazole	125/120 g/L
Sparta	Carbendazim/flusilazole	125/120 g/L
Lyric C	Carbendazim/flusilazole	125/120 g/L
Flusicarb	Carbendazim/flusilazole	125/120 g/L
Early Impact	Carbendazim/flutriafol	150/94 g/L
Propiconazole Plus 250	Carbendazim/propiconazole	125/125 g/L
Propazim	Carbendazim/propiconazole	125/125 g/L
Folicur C	Carbendazim/tebuconazole	133/167 g/L
Rambo	Carbendazim/triadimefon	200/165 g/L
Ranchero 360	Carbendazim/triadimefon	200/165 g/L
Artea	Cyproconazole/propiconazole	80/250 g/L
Endura	Epoxiconazole	125 g/L
Epic	Epoxiconazole	125 g/L
Epoxee	Epoxiconazole	125 g/L
Repute	Triadimenol	250 g/L
Cerix	Epoxiconazole/fluxapyroxad/pyraclostrobin	41.6/41.6 g/L
Abacus	Epoxiconazole/pyraclostrobin	62.5/62.5 g/L
Capitan	Flusilazole	250 g/L
Impact	Flutriafol	125g/L
Ardent	Kresoxim-methyl	500 g/L

### 2.4.3.1 Assessment of resistance

Stakman *et al.* (1962) proposed an infection type (IT) scale on seedlings to standardise disease assessment for rust pathologists around the world. The seedling IT scale was originally used for the assessment of stem and leaf rust of wheat, but was modified to include other cereal rust diseases like *Ph*. Table 2.4 shows the different ITs, their description and host response to *Ph* at the seedling growth stage as proposed by Park and Karakousis (2002). The letters “C” and “N” are included to indicate greater than normal chlorosis or necrosis, respectively (Park *et al.*, 2015).

The symbols “-” and “=” indicate ITs that are lower than normal, whereas “+” and “++” represent higher than normal ITs (Park *et al.*, 2015). Infection types of 3 and higher are considered compatible, implying a virulent pathogen and susceptible host. To correctly describe a phenotype, it is often required to use more than one descriptor, for example, ;12 (Park *et al.*, 2015).

The McNeal scale (McNeal *et al.*, 1971) has been proposed to record and process adult plant disease assessment data. The modified Cobb scale (Peterson *et al.*, 1948) is used as a quantitative measure of disease assessment in adult plants (McIntosh *et al.*, 1995). The latter was originally used for wheat rusts but was consequently applied to other cereal rust diseases. Severity is recorded as a percentage ranging from 0 to 100% infected leaf area (Peterson *et al.*, 1948) in combination with a host response letter. The letters described by Roelfs *et al.* (1992) to measure the host response in adult plants, are as follows: R indicates resistance with or without the presence of necrosis and no uredinia; MR indicates moderate levels of resistance with small uredinia surrounded by either chlorotic or necrotic areas; MS indicates a moderately susceptible response with medium size uredinia surrounded by chlorotic areas; and S indicates susceptibility with large uredinia present with no chlorosis or necrosis. The letter T indicates trace symptoms of infection.

**Table 2.4** Seedling infection type (IT) classes commonly used to rate barley varieties following inoculation with *Puccinia hordei*.

IT	Description	Host
0	No visible symptoms	Immune
;	Fleck	Very R
1	Small uredinia surrounded by necrosis	R
2	Small to medium uredinia surrounded by chlorosis and/or necrosis	R to MR
3	Medium to large uredinia surrounded by chlorosis and/or necrosis	MR to MS
4	Large uredinia without chlorosis and/or necrosis	S
X	Random distribution of variable-sized uredinia	R
C	More than normal chlorosis observed	R
N	More than normal necrosis observed	R
+, ++	Larger than normal uredinia observed	S
-, =	Smaller than normal uredinia observed	S

Host response symbols with R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible

#### 2.4.3.2 Seedling resistance

Flor (1956), examined the genetics of the host-pathogen interaction between flax (*Linum usitatissimum* L.) and the causal pathogen of flax rust *Melampsora lini* (Ehrenb.) Thüm. From the results, it was proposed that for each host gene conferring resistance, there was a complementary avirulence gene in the pathogen which resulted in the gene-for-gene hypothesis for host-pathogen interactions (Table 2.5). Differential lines that contain different resistance genes have since been developed, which allow for determining the avirulence and virulence responses of *Ph* isolates to specific *Rph*-resistance genes (Flor, 1971). This allowed the deployment of especially ASR resistance genes by breeders into new barley varieties against *Ph*.

**Table 2.5** Different interactions possible between a host and pathogen genotype and the resulting disease phenotypes of the host.

Pathogen genotype	Host genotype		
	RR (Resistant)	Rr (Resistant)	rr (Susceptible)
AA (avirulent)	Resistance	Resistance	Disease
Aa (avirulent)	Resistance	Resistance	Disease
aa (virulent)	Disease	Disease	Disease

Singh *et al.* (2001) reported that ASR is expressed during the seedling stage and mostly remains effective to at least some rust pathotypes during a plant's entire growth period. This type of resistance consists of a single major gene that results in a hypersensitive

response (HR) when barley seedlings are infected by a *Ph* isolate that lacks virulence to the specific resistance gene(s) involved (Roane, 1972; Parlevliet, 1976; Parlevliet and Kuiper, 1977; Parlevliet, 1983; Singh *et al.*, 2001; Park and Karakousis, 2002; Park, 2003). The expression of resistance conferred by ASR genes typically involves a rapid cell death around the infection site that stops fungal growth. This is often accompanied by chlorotic flecks which are known as the HR (Singh *et al.*, 2001).

Most of the identified resistance genes to plant pathogens are ASR genes, since they are more easily detected than APR genes and their phenotype determination is more reliable (Manoharachary and Kunwar, 2014). Barley seedlings are usually screened for ASR resistance under controlled environments in greenhouses, which allows the implementation of large-scale screening against different *Ph* isolates in a short period of time (Graner *et al.*, 2000).

Resistance gene deployment requires gene pyramiding through the use of closely linked molecular markers to increase the prospects of durability (Park *et al.*, 2015). Barley varieties listed in Table 2.6 can be used in breeding programmes as donor sources for resistance, as well as to monitor *Ph* variability among isolates. This may include planting these varieties in trap nurseries in the main barley growing areas or screening them against *Ph* isolates under controlled conditions (Graner *et al.*, 2000; Van Niekerk *et al.*, 2001a). Through the deployment of varieties that carry different combinations of rust resistance genes, the probability of mutations and disease occurrence in cereals caused by rust pathogens is likely to decrease (Knott, 1989). This may provide more durable resistance under cultivation for several years within a released variety (Johnson, 1981; 1984).

However, the presence of more than one resistance gene in a differential line may impact the expression of seedling ITs when screened against different *Ph* isolates and complicate the interpretation of results. Therefore, the development of near-isogenic lines (NILs) carrying only one *Rph*-resistance gene in a universal susceptible background, such as the BW introgression barley lines, is important and allows accurate characterisation of resistance gene responses and assist in avirulence/virulence description of *Ph* isolates. The BW introgression lines were developed by backcrossing donor sources of resistance genes

*Rph1* to *Rph15* into the barley variety BW (PI 483237) (Chicaiza *et al.*, 1996; Caffarel, 2005; Martin, 2018; Martin *et al.*, 2020).

Different ASR resistance genes to *Ph* have been mapped at the same chromosome with some being regarded as allelic. Currently, there are three pairs of allelic genes, which include *Rph5/Rph6* on chromosome 3H (Zhong *et al.*, 2003), *Rph9/Rph12* on chromosome 5H (Borovkova *et al.*, 1998) and *Rph12/RphCantala* on chromosome 5HL (Dracatos *et al.*, 2014). The *Rph* genes *Rph15/Rph16* that were previously reported as allelic on chromosome 2H, have recently been reported as the same gene (Weerasena *et al.*, 2004; Fazlikhani *et al.*, 2019; Chen *et al.*, 2020).

#### **2.4.3.3 Partial resistance and adult plant resistance**

Partial resistance was proposed by Parlevliet and Ommeren (1975) to describe resistance to *Ph* in certain barley varieties that were clearly different in expression from ASR and APR. Plants expressing PR produce a susceptible host response at all growth stages, but the infection frequency, latent period, rate of spore production and period of spore production may vary (Park *et al.*, 2015). Partial resistance is quantitative and not based on HR like ASR (Marcel *et al.*, 2007) and the expression thereof is often influenced by several genes (Parlevliet, 1976).

The expression of PR typically hampers haustorium formation, resulting in increased latency periods and will result in a slower rate of disease development (Niks, 1983; Marcel *et al.*, 2007). Barley varieties carrying PR usually exhibit an APR response and, in most cases, the two terms have been associated with each other (Golegaonkar *et al.*, 2009; Park *et al.*, 2015). Partial resistance is generally considered to be a durable form of barley leaf rust resistance (Park *et al.*, 2015). Several PR quantitative trait loci (QTLs) have been described. These include *Rphq2*, *Rphq3* and *Rphq4* that have been identified as consistent QTLs in expressing resistance to *Ph* isolates (Wang *et al.*, 2010). Different QTLs linked to PR have been incorporated into different genetic backgrounds of barley, including the susceptible variety L94 and the variety Vada with known PR to obtain two sets of near-isogenic lines (Wang *et al.*, 2010).

## LITERATURE REVIEW

**Table 2.6** List of barley leaf rust resistance genes (*Rph*) to *Puccinia hordei*, their source (variety) and origin (host plant), whether they provide all-stage (ASR) or adult plant resistance (APR), their chromosomal position and corresponding references.

Gene	Source	Origin	R-type	Position	References
<i>Rph1</i>	Oderbrucker	<i>H. vulgare</i>	ASR	2H	Tuleen and McDaniel, 1971; Tan, 1978
<i>Rph2</i>	Peruvian	<i>H. vulgare</i>	ASR	5HS	Borovkova <i>et al.</i> , 1997; Franckowiak <i>et al.</i> , 1997
<i>Rph3</i>	Estate	<i>H. vulgare</i>	ASR	7HL	Jin <i>et al.</i> , 1993
<i>Rph4</i>	Gold	<i>H. vulgare</i>	ASR	1HS	Tuleen and McDaniel, 1971; Tan, 1978
<i>Rph5</i>	Magnif 104	<i>H. vulgare</i>	ASR	3HS	Tuleen and McDaniel, 1971; Tan, 1978; Mammadov <i>et al.</i> , 2003
<i>Rph6</i>	Boliva	<i>H. vulgare</i>	ASR	3HS	Zhong <i>et al.</i> , 2003
<i>Rph7</i>	Cebada Capa	<i>H. vulgare</i>	ASR	3HS	Brunner <i>et al.</i> , 2000; Graner <i>et al.</i> , 2000
<i>Rph8</i>	Egypt 4	<i>H. vulgare</i>	ASR	Unknown	Tan, 1977
<i>Rph9</i>	HOR2596	<i>H. vulgare</i>	ASR	5HL	Borovkova <i>et al.</i> , 1998
<i>Rph10</i>	Clipper BC8	<i>H. vulgare</i>	ASR	3HL	Feuerstein <i>et al.</i> , 1990
<i>Rph11</i>	Clipper BC867	<i>H. vulgare ssp. spontaneum</i>	ASR	6HL	Feuerstein <i>et al.</i> , 1990
<i>Rph12</i>	Triumph	<i>H. spontaneum</i>	ASR	5HL	Jin <i>et al.</i> , 1993; Borovkova <i>et al.</i> , 1998
<i>Rph13</i>	PI 531849	<i>H. vulgare ssp. spontaneum</i>	ASR	5HL <sup>1</sup>	Jin <i>et al.</i> , 1996; Sun and Neate, 2007
<i>Rph14</i>	PI 584760	Unknown	ASR	2HS	Jin <i>et al.</i> , 1996; Golegaonkar <i>et al.</i> , 2009; Jost <i>et al.</i> , 2020
<i>Rph15</i>	PI 355447	<i>H. vulgare ssp. spontaneum</i>	ASR	2HS	Chicaiza <i>et al.</i> , 1996; Weerasena <i>et al.</i> , 2004
<i>Rph16</i>	HS078/HS084	<i>H. vulgare ssp. spontaneum</i>	ASR	2HS	Ivandic <i>et al.</i> , 1998
<i>Rph17</i>	81882/BSI	<i>H. bulbosum</i>	ASR	2HS	Pickering <i>et al.</i> , 1998
<i>Rph18</i>	88P18/8/1/10	<i>H. bulbosum</i>	ASR	2HL	Pickering <i>et al.</i> , 2000
<i>Rph19</i>	Prior P	<i>H. vulgare</i>	ASR	7HL	Park and Karakousis, 2002
<i>Rph20</i>	Flagship	<i>H. vulgare</i>	APR	5HS	Hickey <i>et al.</i> , 2011
<i>Rph21</i>	Ricardo	<i>H. vulgare</i>	ASR	4H	Sandhu <i>et al.</i> , 2012
<i>Rph22</i>	182Q20	<i>H. bulbosum</i>	ASR	2HL	Johnston <i>et al.</i> , 2013
<i>Rph23</i>	Yerong	<i>H. vulgare</i>	APR	7HS	Singh <i>et al.</i> , 2015

**Table 2.6 (cont.)** List of barley leaf rust resistance genes (*Rph*) to *Puccinia hordei*, their source (variety) and origin (host plant), whether they provide all-stage (ASR) or adult plant resistance (APR), their chromosomal position and corresponding references.

Gene	Source	Origin	R-type	Position	References
<i>Rph24</i>	ND24260-1	<i>H. vulgare</i>	APR	6H	Ziems <i>et al.</i> , 2017
<i>Rph25</i>	Fong Tien	<i>H. vulgare</i>	ASR	5HL	Kavanagh <i>et al.</i> , 2017
<i>Rph26</i>	Emir	<i>H. bulbosum</i>	ASR	1HL	Yu <i>et al.</i> , 2018
<i>Rph27</i>	Quinn	<i>H. vulgare</i>	ASR	4HS	Rothwell <i>et al.</i> , 2020
<i>Rph28</i>	Barke	<i>H. vulgare</i> ssp. <i>Spontaneum</i>	ASR	5H	Mehnaz <i>et al.</i> , 2021

<sup>1</sup>*Rph13* was previously mapped to chromosome 5H (Jin *et al.*, 1996) while recently mapped to chromosome 3H (Jost *et al.*, 2020)

Consequently, six QTLs were found, namely *Rphq1*, *Rphq2*, *Rphq3*, *Rphq4*, *Rphq5* and *Rphq6* contributing to PR of barley variety Vada (Wang *et al.*, 2010). From the six QTLs, only three were successfully mapped, namely *Rphq2*, *Rphq3* and *Rphq4* to chromosomes 2H, 6H and 5H respectively (Wang *et al.*, 2010). *Rphq2* was found to be effective in seedlings, *Rphq3* in both seedlings and adult plants and *Rphq4* only in adult plants (Wang *et al.*, 2010). Deploying PR sources to rust pathogens has become the preferred breeding method in genetic control of cereal rusts (Chu *et al.*, 2009). This may involve the stacking of PR with other resistance sources using closely linked molecular markers like Restriction Fragment Length Polymorphisms (RFLPs) (Botstein *et al.*, 1980), Amplified Fragment Length Polymorphisms (AFLPs) (Vos *et al.*, 1995), Single Nucleotide Polymorphisms (SNPs) (Brookes, 1999) and Diversity Arrays Technology (DArT) markers (Jaccoud *et al.*, 2001). In SA the efficiency of PR sources to *Ph* has not been determined.

All APR genes were first identified as PR genes and were later renamed. For example, *Rph20* was first identified as *Rphq4* in the variety Vada (Qi *et al.*, 1998). Golegaonkar *et al.* (2010) characterised *Rphq4* in the variety Pompadour which was later mapped to chromosome 5H and designated as *Rph20* by Hickey *et al.* (2011). The APR resistance gene *Rph23* was found closely linked to molecular marker Ebmac0603 associated with *qRph-Yer-7H* and is present in some Australian varieties (Singh *et al.*, 2015). The designated APR gene *Rph24* was mapped to chromosome 6H by Ziemis *et al.* (2017). This APR gene was first named *Rphq3* at the seedling stage for PR after being detected in variety Vada (Qi *et al.*, 1998) and later renamed as *qRphND* (Qi *et al.*, 1998; Castro *et al.*, 2012; Gonzalez *et al.*, 2012) following a study involving barley variety ND24260-1 (Hickey *et al.*, 2011).

According to Smit and Parlevliet (1990), varieties with APR genes show susceptibility during the seedling growth stages but as the plants mature into the reproductive growth stages, there is a reduction in the number and size of rust pustules. Trials to determine the host response of APR in barley varieties are normally conducted under field conditions including different environments and years (Singh *et al.*, 2013) to ensure reliability and validity of the data. However, even under field conditions, many limitations exist, preventing the rapid characterisation and deployment of APR genes in resistance breeding (Dracatos *et al.*, 2015). These may include variation in disease pressure and environmental conditions like

the temperature at the same locality between seasons (Riaz *et al.*, 2016; Rothwell *et al.*, 2019). This poses a major challenge to discover and characterise especially APR sources with moderate levels of *Ph* resistance (Rothwell *et al.*, 2019).

Greenhouse based screening of adult plants was proposed to overcome the limitations experienced with seasonal field screening, preventing the slow process of identifying APR genes when compared to ASR (Rothwell *et al.*, 2019). This was successfully implemented for wheat screening against *Pt* (Pretorius *et al.*, 2000; Riaz *et al.*, 2016) and barley screening against net form net blotch caused by *Pyrenophora teres* f. sp. *teres*, (*P. teres* Drechsler; anamorph *Drechslera teres* [Sacc.] Shoem.) (Wallwork *et al.*, 2016).

Rothwell *et al.* (2019) reported an additive APR effect observed in Lenka (*Rph20* + *Rph23* + *Rph24*) and Henly (*Rph20* + *Rph24*) when compared with the resistance response of Flagship (*Rph20*). This response was demonstrated by Ziems *et al.* (2017) for *Rph20* and *Rph24* and was in further support of Rothwell's findings (Rothwell *et al.*, 2019). However, a contradicting result indicated that the combination of all three APR genes somehow resulted in lower levels of resistance compared with only barley varieties carrying *Rhp20* and *Rph24* (Ziems *et al.*, 2017). This may indicate that *Rph20* masks the interaction between *Rph23* and *Rph24* (Rothwell *et al.*, 2019). Lower temperatures of 18°C indicate greater resistance compared to higher temperatures of 23°C when screening for resistance expressed by APR genes. This emphasizes the importance of temperature in the expression of APR genes which was also reported for the *Lr34* resistance gene in wheat (Singh and Gupta, 1992). Greenhouse and field testing of barley germplasm led to the identification of APR in barley varieties in Australia, China, Spain and Uruguay (Park *et al.*, 2015). To date, similar results have not been published for SA.

## 2.5 Molecular markers

Molecular markers can provide an estimation of the genetic variation of individuals in a population. Furthermore, they can be used to locate genes controlling different traits in an individual (Singh *et al.*, 2011). These markers are in the non-coding regions of Deoxyribonucleic acid (DNA) and are not influenced by environmental conditions or the growth stage of the individual (Winter and Kahal, 1995).

There are various molecular marker techniques available for mapping and pyramiding of *Rph*-resistance genes in barley. These include AFLPs, RFLPs, Random Amplified Polymorphic DNA (RAPD) and the commonly used microsatellite marker systems (Mehnaz et al., 2021) which were successfully used to map *Rph2* (Borovkova et al., 1997; Franckowiak et al., 1997), *Rph15* (Weerasena et al., 2004), *Rph19* (Park and Karakousis, 2002) and *Rph21* (Sandhu et al., 2012). New techniques such as DArTs and SNPs rely on Next-Generation Sequencing (NGS) and Genotyping by Sequencing (GBS) technologies. These techniques are faster, more affordable and have been used to map *Rph14*, *Rph9/Rph12*, *Rph25*, *Rph27* and *Rph28* resistance genes (Golegaonkar et al., 2009; Dracatos et al., 2014; Kavanagh et al., 2017; Rothwell et al., 2020; Mehnaz et al., 2021).

Knowledge about the genetic variation of the pathogen population is essential to understand the genetic diversity and the relationship between the pathogen and its host (Aradhya et al., 2001). Historically these studies involved mostly annual rust surveys to monitor cereal rust populations in terms of their avirulence and virulence to a predetermined set of resistance genes. More recently however, this also included the genotyping of cereal rust isolates using microsatellites as DNA markers. These are codominant, PCR-based molecular markers (Kolmer et al., 2019). Microsatellite markers are inherited based on Mendelian laws and generate essential genetic information (Liu and Kolmer, 1998). Several recent papers reported on the genetic diversity among isolates of cereal rust pathogens in SA using microsatellite markers (Visser et al., 2012; Pretorius et al., 2015; Boshoff et al., 2019). However, none of these studies involved *Ph*, despite the importance of this pathogen. In a recent study, 13 microsatellite markers were used to determine the genetic variation among isolates of the oat stem rust pathogen in SA (Boshoff et al., 2019). Isolates of *P. graminis* Pers. Pers. f. sp. *avenae* Eriks. & E. Henn. were collected from diverse locations in SA. The genotypic data was used to determine the extent of genetic variation between isolates, to determine whether any correlation exists between the genotype and phenotype of isolates and to confirm the asexual status of the pathogen in SA.

A study by Karaoglu and Park (2014) reported on the isolation and characterisation of 19 *Ph* isolates using 76 polymorphic microsatellite markers. The markers displayed high

polymorphism and genetic variability between the isolates. A conclusion was made that the markers have the capacity to discriminate between isolates of barley leaf rust caused by *Ph*. This provided a useful application for molecular markers when conducting genotyping between *Ph* isolates for population genetics.

## **2.6 Conclusions**

Barley leaf rust is largely controlled through the application of fungicides, involving the combination of a seed treatment followed by foliar applications, in the WC production region of SA. Currently, there is a lack of information on the number of *Ph* races, their avirulence/virulence profiles, as well as the extent of genotypic variation among isolates in SA. This prevents the accurate characterisation of local barley varieties for their response to *Ph* in seedling and adult plant stage studies. Furthermore, international barley lines carrying designated *Rph* sources of resistance, need to be characterised locally before they can be considered for use in resistance breeding programmes. Molecular markers closely linked to some of these resistance sources are available and if found informative in local germplasm lines, their use will assist in the more rapid release of adapted barley varieties with resistance to *Ph*.

## Chapter 3 Materials and Methods

### 3.1 Materials

#### 3.1.1 Phenotyping of *Puccinia hordei* isolates

Two historic *Ph* control isolates were obtained from Dr Tarekegn Terefe from the Agricultural Research Council, Small Grains, (ARC-SG) Bethlehem. These isolates represented two previously described *Ph* races dating back to 1994 (SAPh 3231, renamed UVPPh3231) and 1998 (SAPh 7231, renamed UVPPh7231), respectively (Van Niekerk *et al.*, 2001a). The third *Ph* control isolate was collected on barley trial plots planted outside Greytown, KZN during the 2015 growing season (ZA Pretorius, unpublished data).

Barley leaf samples infected with *Ph* were submitted by co-workers during the 2017 (7 samples), 2018 (16) and 2019 (17) growing seasons from the south WC province. Samples submitted included infected barley leaves from commercial fields collected near Klipdale, Voorstekop, and the Hemel and Aarde Valley, as well as from barley breeders' plots planted near Riviersonderend (Tygerhoek, Department of Agriculture, WC), Caledon (Roodebloem, ABInBev) and Napier (Sensako, now Syngenta) experimental farms (Appendix 1).

Barley differential line seeds were obtained from Prof Robert Park and Dr Davinder Singh (Plant Breeding Institute, The University of Sydney, Australia), Dr Paul Johnson (Plant and Food Research, Lincoln, New Zealand) and Prof Brian Steffenson (Department of Plant Pathology, University of Minnesota, St Paul, MN 55108, USA). Seed of South African barley varieties was provided by Mr Daniel de Klerk (ABInBev, Caledon, SA).

#### 3.1.2 Genotyping of *Puccinia hordei* isolates

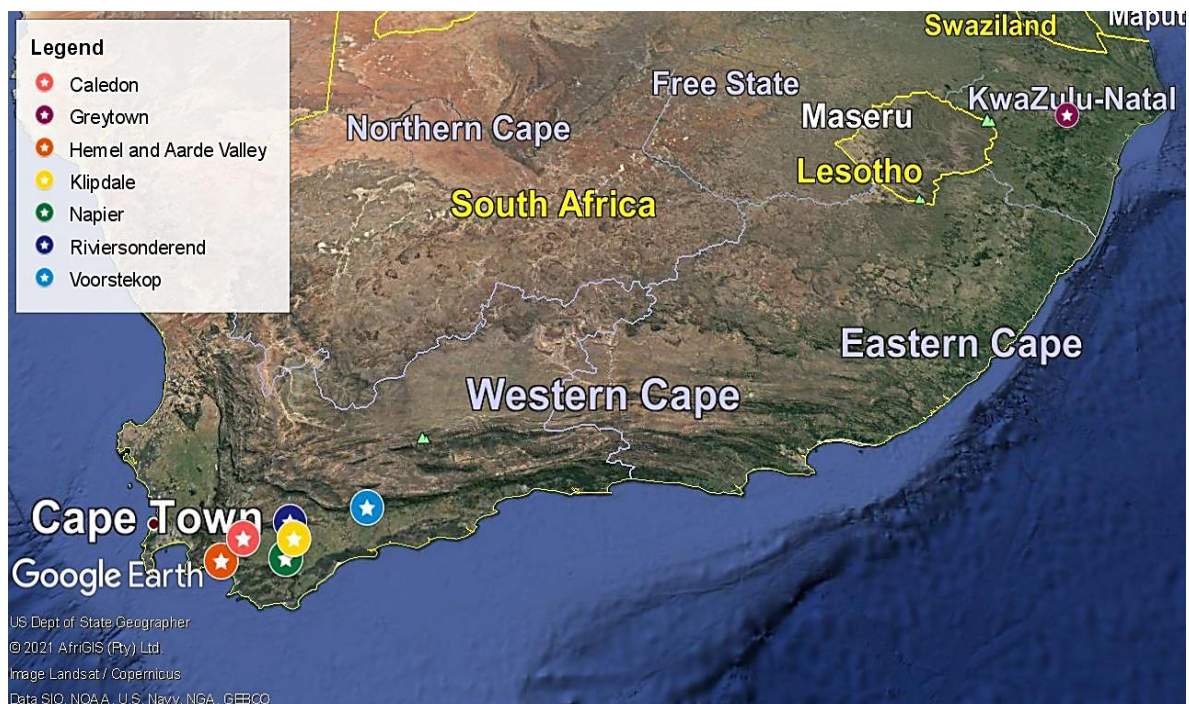
In total, 48 single-pustule *Ph* isolates (Appendix 1) were genotyped in the study. Barley variety Gus leaves containing one or two uredinia were cut from infected seedlings, placed in 2 mL Eppendorf tubes and stored at -80°C.

## 3.2 Methods

### 3.2.1 Phenotyping of *Puccinia hordei* isolates

#### 3.2.1.1 Collection of *Puccinia hordei* isolates

Urediniospores were collected from *Ph* infected barley leaves sampled from varieties planted under dryland conditions in the WC during the 2017, 2018 and 2019 growing seasons (Figure 3.1; Appendix 1). When more than one sample was collected at the same locality in a particular year, they were representative of the different barley varieties planted at that locality. Field samples were preserved by placing infected barley leaves into glycine crossing bags. Samples were dried for 48 h at room temperature before being mailed to the Plant Pathology rust laboratory at the University of the Free State. Upon arrival, urediniospores were collected from infected barley leaves into size 00 gelatine capsules by connecting an air vacuum (Vacuubrand® pump-model MZ2) to cyclone spore collection devices (Pretorius *et al.*, 2019), after the capsules were stored in Cryo.s™ tubes at -80°C.



**Figure 3.1** Localities in South Africa where *Puccinia hordei* infected leaf samples were collected from.

Spore collectors were cleaned by dipping them into acetone for 60 s followed by a 2 h heat treatment at 75°C before re-use. Isolates were numbered with the initial *Ph* followed by a numeric isolate number, an abbreviation for the locality of collection and the year of collection.

### **3.2.1.2 *Puccinia hordei* race typing**

Barley seeds from the universal susceptible variety Gus were planted in 10 cm diameter plastic pots filled with Mikskaar Professional Potting Soil 70 (Hygrotech, Pretoria, SA) and watered daily with reverse osmosis purified water. Seedlings were treated with 50 mL (per pot) of a 0.6% (w/v) maleic hydrazide solution (Reagent Plus®, Sigma-Aldrich) once coleoptiles emerged, to retard secondary leaf development. Seedlings were fertilized with Multifeed-classic water-soluble fertiliser [Effekto Multifeed® Classic, NPK analysis 19:8:16 (43), 2.5 g/L concentration] as per manufacturer's instructions once before and once after inoculation. Iron (Fe) in the form of Fe chelate (Wonder™, 130 g/kg Fe, 0.6 g/L concentration) was applied according to the manufacturers recommendations to prevent excessive yellowing of the primary leaves. Inoculation was done 8 days after planting. Before inoculation, the stored field isolates were retrieved from the -80°C freezer and heat shocked at 46°C for 6 min. Gus seedlings were spray inoculated using a pressure pump (Vacuubrand® pump- model MZ2) at 25 kPa pressure setting connected to an inoculation device with a suspension of urediniospores in 300 µL Soltrol® 130 isoparaffinic oil. Inoculations were conducted in a closed room within an enclosed booth that was flushed for 60 s between sprays with water to prevent cross contamination of isolates.

Inoculated seedlings were dried in growth cabinets (200 µE/m<sup>2</sup>/sec light; 25°C) for 1 h and then placed in the dark in a dew stimulation chamber at 18°C (±1°C) for 18 h. Upon removal from the dew chamber, seedlings were dried off in growth cabinets for 2 h (conditions as above) and then placed in separate compartments in a greenhouse. After 12 days, single-pustules were collected for each field isolate and subsequently increased through re-inoculation onto Gus seedlings following the procedures described above. A single-pustule isolate number was added to the initial isolate number for each field isolate. Seven single-pustules isolates were established from field samples collected during 2017, 32 from 2018 and 35 from 2019, respectively. Controls included two isolates representative of the two

previously described races UVPh3231 and UVPh7231, as well as the isolate collected on barley outside Greytown, KZN (Ph3\_Gt2015) (Appendix 1).

Pathotyping proceeded by inoculating urediniospores ( $\pm 1$  mg/mL in 800  $\mu$ L Soltrol<sup>®</sup> 130 isoparaffinic oil) from the single-pustule derived isolates on a standard barley differential set. The barley differential lines consisted of 28 entries with known *Rph*-resistance genes and the susceptible controls Gus and PI 532013 (Table 3.1). Differential lines were planted as six clumps of seed ( $\pm 5$  seeds per clump) per 10 cm diameter pot filled with Mikskaar potting soil. Procedures for seedling growth, inoculation and incubation were as described above but seedlings were not treated with maleic hydrazide. Seedlings were kept after inoculation in a greenhouse cubicle at a night/day temperature regime of 18°C and 21°C, respectively with an average photosynthetic photon flux density of 1,350  $\mu$ mol/(m<sup>2</sup>.s) measured at plant level at midday using an Apogee MQ-500 quantum meter. After 12 days, the seedlings were scored using a 0 to 4 scale (Table 3.2; Park and Karakousis, (2002)). Seedling ITs of 0 to 2+ and X (random distribution of variable-sized uredinia) were characterised as low, while ITs of 3 to 4 were interpreted as high. In addition, a representative isolate of each of the *Ph* races was used to determine seedling ITs for the BW introgression lines containing the resistance genes *Rph1* to *Rph15* (Table 3.3).

### 3.2.1.3 Naming of *Puccinia hordei* races

Seedling IT data recorded on the differential set for each isolate was used to allocate octal values (1 to 4 000) as previously denoted by Gilmour (1973) and recently depicted in Elmansour *et al.* (2017) using the designated octal race system. The 12 differential lines (Sudan, Peruvian, Estate, Gold, Magnif 104, Bolivia, Cebada Capa, Egypt 4, Abyssinian, Clipper BC8, Clipper BC867 and Triumph) used in race naming, their respective resistance genes (*Rph1*; *Rph2*; *Rph3*; *Rph4*; *Rph5*; *Rph2+Rph6*; *Rph7*; *Rph8*; *Rph9*; *Rph10*; *Rph11* and *Rph12*) and allocated octal values (1, 2, 4, 10, 20, 40, 100, 200, 400, 1 000, 2 000 and 4 000) are listed in Table 3.1. This resulted in the opportunity to allocate a unique race name to each isolate based on their avirulence/virulence profile on the 12 differentials. Octal values were added when virulence (seedling ITs  $\geq 3$ ) was recorded and the sum calculated over the 12 lines to provide a digit code. In this study, the applied race naming system included

the prefix UV for University of the Free State, adding *Ph* and the appropriate sum of the octal values to name isolates into races.

**Table 3.1** Barley differential lines, their reported resistance (*Rph*) gene(s), and octal value.

Differential line <sup>1</sup>	<i>Rph</i> gene(s) <sup>5</sup>	Octal value
Gus <sup>2</sup>	Nil	
PI 532013 <sup>3</sup>	Nil	
Sudan <sup>2</sup>	<i>Rph1</i>	1
Berg <sup>2</sup>	<i>Rph1</i>	
Peruvian <sup>2</sup>	<i>Rph2</i>	2
Gatam <sup>2</sup>	<i>RphGatam</i>	
Reka 1 <sup>2</sup>	<i>Rph2+Rph19</i>	
Ricardo <sup>2</sup>	<i>Rph2+Rph21</i>	
Estate <sup>2</sup>	<i>Rph3</i>	4
Gold <sup>2</sup>	<i>Rph4</i>	10
Quinn <sup>2</sup>	<i>Rph2+Rph5+Rph27</i>	
Magnif 104 <sup>2</sup>	<i>Rph5</i> <sup>5</sup>	20
Bolivia <sup>2</sup>	<i>Rph2+Rph6</i>	40
Bolivia/Gus RIL <sup>2</sup>	<i>Rph6</i>	
Cebada Capa <sup>2</sup>	<i>Rph7</i>	100
Egypt 4 <sup>2</sup>	<i>Rph8</i>	200
Abyssinian <sup>2</sup>	<i>Rph9</i>	400
Clipper BC8 <sup>2</sup>	<i>Rph10</i>	1 000
Clipper BC867 <sup>2</sup>	<i>Rph11</i>	2 000
Triumph <sup>2</sup>	<i>Rph12</i>	4 000
PI 531849 <sup>2</sup>	<i>Rph13</i>	
PI 584760 <sup>2</sup>	<i>Rph14</i>	
HOR 1063 <sup>2</sup>	<i>Rph16 (Rph15)</i>	
81882 <sup>4</sup>	<i>Rph17</i>	
38P18/8/1/10 <sup>4</sup>	<i>Rph18</i>	
Prior (P) <sup>2</sup>	<i>Rph19</i>	
Ricardo/Gus RIL <sup>2</sup>	<i>Rph21</i>	
182Q20/1/M1 <sup>4</sup>	<i>Rph22</i>	
Fong Tien <sup>2</sup>	<i>Rph25</i>	
200A12/8/2/M2 <sup>4</sup>	<i>Rph26</i>	

<sup>1</sup>Differential lines, *Rph* genes and octal values according to Ivandic *et al.* (1998); Pickering *et al.* (1998); Pickering *et al.* (2000); Sandhu *et al.* (2012); Johnston *et al.* (2013); Elmansour *et al.* (2017); Kavanagh *et al.* (2017)

<sup>2</sup>Seed provided by Prof Robert Park (Plant Breeding Institute, The University of Sydney, Australia); <sup>3</sup>Prof Brian Steffenson (Department of Plant Pathology, University of Minnesota, St Paul, MN 55108, United States of America) and <sup>4</sup>Dr Paul Johnson (Plant and Food Research, Lincoln, New Zealand)

<sup>5</sup>*Rph5/Rph6* (Zhong *et al.*, 2003), *Rph9/Rph12* (Borovkova *et al.*, 1998) and *Rph15/Rph16* (Weerasena *et al.*, 2004) were reported to be allelic. *Rph15/Rph16* are now reported as the same gene (Chen *et al.*, 2020)

MATERIALS AND METHODS

**Table 3.2** Seedling infection type (IT) scale, associated host response, and a description of the corresponding visible symptoms used in the phenotypic characterisation of single-pustule isolates of *Puccinia hordei*.

IT value/letter <sup>1</sup>	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 necrosis or chlorosis
3	Moderately susceptible	Medium sized uredinia with or without chlorosis
4	Susceptible	Large uredinia without chlorosis
X	Resistant	Heterogeneous, similarly distributed uredinia over the leaves

<sup>1</sup>Infection types are based on “0” to “4” scale proposed by Park and Karakousis (2002)

**Table 3.3** Bowman (BW) barley leaf rust introgression lines and their reported *Rph*-resistance gene and *Rph* allele.

BW line <sup>1,2</sup>	<i>Rph</i> gene <sup>3</sup>	<i>Rph</i> allele
BW682	<i>Rph1</i>	<i>Rph1.a</i>
BW743	<i>Rph2</i>	<i>Rph2.t</i>
BW746	<i>Rph3</i>	<i>Rph3.c</i>
BW752	<i>Rph4</i>	<i>Rph4.d</i>
BW755	<i>Rph5</i>	<i>Rph5.e</i>
BW756	<i>Rph6</i>	<i>Rph5.f</i>
BW758	<i>Rph7</i>	<i>Rph7.g</i>
BW759	<i>Rph8</i>	<i>Rph8.h</i>
BW760	<i>Rph9</i>	<i>Rph9.i</i>
BW683	<i>Rph10</i>	<i>Rph10.o</i>
BW684	<i>Rph11</i>	<i>Rph11.p</i>
BW761	<i>Rph12</i>	<i>Rph9.z</i>
BW685	<i>Rph13</i>	<i>Rph13.x</i>
BW686	<i>Rph14</i>	<i>Rph14.ab</i>
BW719	<i>Rph15</i>	<i>Rph15.ad</i>

<sup>1</sup>Bowman lines, *Rph* genes and *Rph* alleles as reported in Martin (2018)

<sup>2</sup>Seed provided by Prof Brian Steffenson (Department of Plant Pathology, University of Minnesota, St Paul, MN 55108, United States of America)

<sup>3</sup>*Rph5/Rph6* (Zhong *et al.*, 2003), *Rph9/Rph12* (Borovkova *et al.*, 1998) and *Rph15/Rph16* (Weerasena *et al.*, 2004) were reported to be allelic. *Rph15/Rph16* are now reported as the same gene (Chen *et al.*, 2020)

### 3.2.2 Greenhouse and field screening of barley varieties

#### 3.2.2.1 Greenhouse screening

The seedling responses of four barley varieties that carry designated APR genes (Table 3.4), as well as 10 South African barley varieties (Table 3.5), were determined in replicated greenhouse trials to representative isolates of *Ph* races UVPh3231, UVPh7231 and UVPh7235. Susceptible controls included were the varieties Gus and PI 532013. Pre- and post-inoculation treatments of seedlings were done as described in section (3.2.1.2). The seedling ITs were assessed 12 days after inoculation as described in Table 3.2. The trial was repeated to confirm all seedling responses.

Greenhouse trials were further established to determine the *Ph* adult plant response of selected barley varieties. Included were the BW introgression set of 15 barley lines (Table 3.3), four barley varieties with known *Ph* APR genes (Table 3.4), and 10 South African varieties (Table 3.5).

**Table 3.4** Barley varieties and their reported adult plant resistance (*Rph*) gene(s).

Variety <sup>1</sup>	<i>Rph</i> gene <sup>2</sup>
Yerong	<i>Rph2+Rph23</i>
Lenka	<i>Rph20+Rph23+Rph24</i>
Baronesse	<i>Rph20+Rph24</i>
Flagship	<i>Rph20</i>

<sup>1</sup>Seed of barley varieties was provided by Mr Daniel de Klerk (ABInBev, Caledon, South Africa) and Dr Davinder Singh (Plant Breeding Institute, The University of Sydney, Australia)

<sup>2</sup>*Rph* genes as reported in Borovkova *et al.* (1997); Hickey *et al.* (2011); Singh *et al.* (2015); Ziems *et al.* (2017)

**Table 3.5** List of South African barley varieties included in this study and their production region.

Variety <sup>1</sup>	Barley production region
Agulhas	WC dryland
Cristalia	Summer rainfall irrigation
Deveron	Summer rainfall irrigation
Elim	WC dryland
Erica	WC dryland
Genie	Summer rainfall irrigation
Hessekwa	WC dryland
Kadie	WC dryland
KWS Irina	Summer rainfall irrigation
Overture	Summer rainfall irrigation

<sup>1</sup>Seed of barley varieties was provided by Mr Daniel de Klerk (ABInBev, Caledon, South Africa)

In all trials, the two varieties Gus and PI 532013 were included as susceptible controls. Five seeds of each variety were planted in 1.8 L capacity pots, six pots per variety, filled with steam-sterilised soil. The procedure included two pots per variety for each of the three *Ph* races (UVP<sub>h</sub>3231, UVP<sub>h</sub>7231 and UVP<sub>h</sub>7235). Plants were watered daily with reverse osmosis purified water and fertilised weekly with 0.2% (w/v) Multifeed-Classic water-soluble fertiliser [Effekto<sup>®</sup>, NPK Analysis 19:8:16 (43), 2.5 g/L concentration] at 50 mL per pot. Inoculations proceeded when the varieties reached Zadoks growth stages 49 to 61 (first awns visible to early flowering) (Zadoks *et al.*, 1974) in the greenhouse. Urediniospores of the *Ph* isolates, representative of each race, were increased on the susceptible variety Gus prior to inoculation. Freshly collected urediniospores were suspended in reverse osmosis water containing Tween20<sup>®</sup> (0.03% v/v) to a final spore concentration of ±1 mg/mL. Using a low-pressure spray-gun, connected to a compressor set at 250 kPa, 100 mL of the spore concentration for each *Ph* race isolate was applied to

mainly the flag leaves of plants in 10 pots at a time as uniformly as possible. Application equipment was carefully cleaned after inoculation with each *Ph* race isolate to prevent contamination. Inoculated plants were placed in a dew simulation chamber at 18°C ( $\pm 1^\circ\text{C}$ ) for 18 h. Following incubation, the plants were placed in a greenhouse cubicle with post-inoculation treatments as described above in section (3.2.1.2). Fifteen days following inoculation, the flag leaves of each entry were scored phenotypically and the host response determined using infection response types including R = Resistant, MR = Moderately Resistant, MS = Moderately Susceptible and S = Susceptible (Roelfs *et al.*, 1992).

### **3.2.2.2 Field screening**

The field response of the barley differential lines and varieties was determined in screening nurseries on the Corteva™ Research Farm near Greytown, KZN, SA during the 2017, 2018 and 2019 growing seasons. Trials were established annually during the 3<sup>rd</sup> week of June by planting the respective differential lines and varieties in 1 m observation rows, with a 50 cm inter-row spacing. Regular irrigation and sufficient fertiliser were applied to support optimum plant development. Planting of the susceptible barley varieties Gus and PI 532013 was repeated every 10 rows. Early infection was initiated annually about six weeks after planting, by spraying urediniospores of race UVP<sub>h</sub>7235 onto barley rows planted with the two susceptible control varieties. Urediniospores were suspended in Soltrol® 130 isoparaffinic oil ( $\pm 3$  mg/mL) and then sprayed onto the plants using an ultralow-volume sprayer (ULVA; Micron Group, Bromyard, England). Following the first signs of successful infection, no further inoculations were carried out and further infection and spread of *Ph* were allowed to develop naturally. The field response for the barley varieties was determined by severity (% flag leaf area infected) using the modified Cobb scale (Peterson *et al.*, 1948), combined with host infection response types as described above in section (3.2.2.1).

In addition, barley trials were established under rainfed conditions on the Sensako Research Farm near Napier, WC during the 2020 season. Trial entries consisted of a replicate of the Greytown trial. The trial was established during the first week of June with trial entries planted in 1 m observation rows. Fertiliser and pest control were applied according to the Sensako protocol and no artificial inoculations were carried out. Leaf

samples obtained from trial entries were used to confirm the *Ph* race that dominated through natural infection. *Ph* assessment was done on the flag leaves as described above when the disease reached 100% on the susceptible control varieties.

### 3.2.3 Genotyping of *Puccinia hordei* isolates

#### 3.2.3.1 DNA extraction

Upon removal from -80°C storage, leaf samples were freeze-dried for two days and ground to a fine powder for 1 min using a TissueLyser (Qiagen, Hilden, Germany). Genomic DNA was extracted from ground leaf tissue using the cetyl trimethylammonium bromide (CTAB) method of Saghai-Marooof *et al.* (1984) as outlined by Visser *et al.* (2009). After resuspending 100 mg ground leaf tissue in 750 µL extraction buffer [100 mM Tris(hydroxymethyl) aminomethane hydrochloride (Tris-HCl) pH 8.0, 20 mM ethylenediaminetetraacetic acid (EDTA), 1.4 M NaCl, 2% (w/v) CTAB], the suspension was incubated for 1 h at 65°C. The suspension was extracted once with 500 µL chloroform (ChCl<sub>3</sub>)/isoamyl alcohol (IAA) (24:1, v/v), mixed and centrifuged at 12 000 *g* for 10 min at 4°C. Genomic DNA was precipitated from the aqueous phase with 500 µL isopropanol and incubated at room temperature for 20 min. After centrifugation at 12 000 *g* for 10 min at 4°C, the DNA pellet was washed once with 500 µL ice cold 70% (v/v) ethanol for 20 min at room temperature before centrifugation at 12 000 *g* for 5 min at 4°C. DNA pellets were air-dried for 1 h at room temperature and dissolved overnight at 4°C in 200 µL TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA).

Complete removal of RNA was done by adding RNase to a final concentration of 100 µg/mL and incubating it for 2 h at 37°C. The DNA was again extracted with 200 µL ChCl<sub>3</sub>/IAA (24:1, v/v) and 20 µL 7.5 M ammonium acetate, and then centrifuged at 12 000 *g* for 5 min at 4°C. The DNA was precipitated from the aqueous phase for 2 h at -20°C with 500 µL ice-cold 100% (v/v) ethanol and centrifuged at 12 000 *g* for 15 min at 4°C. The DNA pellet was washed once with 500 µL ice-cold 70% (v/v) ethanol, centrifuged at 12 000 *g* for 10 min at 4°C, air-dried and finally dissolved overnight in 50 µL TE buffer at 4°C.

The concentration of all DNA samples was determined using the NanoDrop™ 2000 (Thermo Scientific™) spectrophotometer. DNA purity was expressed as the  $A_{260}/A_{280}$  and  $A_{260}/A_{230}$  ratios. The DNA samples were finally diluted to 30 ng/ $\mu$ L with TE.

### **3.2.3.2 Agarose gel electrophoresis**

To confirm the DNA quality, 200 ng genomic DNA of each sample was separated on a 1.2% (w/v) agarose gel prepared in 0.5x TAE (20 mM Tris-acetate pH 8.0, 0.5 mM EDTA), also using 0.5x TAE as running buffer (Sambrook *et al.*, 1989). DNA was mixed with loading buffer [15% (w/v) ficoll, 0.25% (w/v) bromophenol blue] in a 2.5:1 (v/v) ratio and loaded on the gel. DNA separation was at 10 V/cm for 20 min and the image was captured with the Gel Doc™ EZ System (Bio-Rad Laboratories).

### **3.2.3.3 Microsatellite analysis of *Puccinia hordei* isolates**

Genotyping of 48 *Ph* isolates (Appendix 1) was done using 20 microsatellite markers described by Karaoglu and Park (2014) (Table 3.6). Each single 10  $\mu$ L PCR reaction consisted of 2 ng DNA, 1  $\mu$ M of each primer set and a 1x concentration of KapaTaq ReadyMix (Sigma-Aldrich, USA). The PCR regime included an initial 2 min denaturation step at 94°C, followed by 40 cycles of 94°C for 30 s, 59°C for 30 s and 72°C for 1 min. A final 10 min elongation step at 72°C was included. To verify positive PCR amplification, 2  $\mu$ l of each PCR reaction was mixed with 4  $\mu$ l loading buffer [15% (w/v) ficoll, 0.25% (w/v) xylene cyanol] and separated on a 1.2% (w/v) agarose gel as described above in section (3.2.3.2).

### **3.2.3.4 Microsatellite allele separation**

The amplified alleles were separated on the QIAxcel Advanced system (QIAGEN, Germany) using a QX DNA High Resolution cartridge. Separation was done using the OM800 method with a 5 kV sample injection voltage, 10 s injection time, 6 kV separation voltage with a total 920 s separation time. Allele sizes were determined using the QX 15 bp/1 kb alignment marker and QX 50-800 bp v. 2.0 DNA size marker according to the manufacturer's instructions. The allelic data matrix for *Ph* was prepared as for a diploid organism due to the dikaryotic nature of *Ph*. The acquired data was finally hand-edited according to the length of the microsatellite repeat motif (Karaoglu and Park, 2014).

**Table 3.6** Microsatellite markers used for the genotypic analysis of South African *Puccinia hordei* isolates.

Marker	Locus	Repeat motif	Forward primer (5'-3')	Reverse primer (5'-3')	Size (basepairs)
Ph18	SUNPh9-37	(CAAAAG) <sub>9</sub>	CATTGGCTGGATATATAGAC	AAAATCCTGCAAATCTTTCC	220-320
Ph19	SUNPh9-46	(TTTTCT) <sub>9</sub>	AAATGCCAATCATAATTCAG	TGAGTCGGTCATTAAGAAG	240-310
Ph22	SUNPh10-42	(AAAAT) <sub>19</sub>	CATGTCTAATTTCTGTTTTG	AGAGGGGAAAAAATGAAGTG	240-290
Ph23	SUNPh10-44	(AAAAC) <sub>25</sub>	GGGTCCCTTTGGTTTATATT	TCCCACAGTTCATTCTAAA	224-312
Ph24	SUNPh1-28	(TTTCT) <sub>7</sub>	AGCCCCGGGAGATAACAATAA	GGCGCCCTGCTGTTGGATAC	180-210
Ph25	SUNPh1-42	(TTTTTCC) <sub>5</sub>	ACAGGAGTGGTCGAAGTTCT	GTGGTTGGTGGAAGGATAAT	190-223
Ph27	SUNPh7-31	(GAAA) <sub>7</sub>	ATGCGAGGTCAAGTGTTTAG	GCGTCTCCCCTGTGAATATC	244-278
Ph28	SUNPh7-36	(TGTA) <sub>7</sub>	TCCCCGACAGATTCGTTTTT	TTCGACGATCATAATATCCT	245-268
Ph29	SUNPh7-38	(TTGT) <sub>7</sub>	TCTGTGTTTTGTCCAATAAT	AGGCCTAACTGGGAACTGAA	228-260
Ph31	SUNPh8-30	(AAAGG) <sub>9</sub>	GAGTGGGTGTTTTGTTTG	ATGTTTGGGGATGTTCTTTT	200-210
Ph32	SUNPh9-07	(AAAAAG) <sub>7</sub>	GGTAAGGGGTTAGTTAATCT	CAACCACAACAATTATTAGA	280-298
Ph34	SUNPh10-16	(TTTC) <sub>8</sub>	TTCCAGGTTAGTGTTCCTC	GCTCCTCAGTTATAGTCCTC	196-208
Ph36	SUNPh10-28	(CAAT) <sub>13</sub>	TCGTCGGTTAGAAAGAATAG	AGAGGATGGGGATGTTTTTG	280-290
Ph37	SUNPh10-30	(AAAG) <sub>15</sub>	GTCGATACGCCTGAATATTG	TACGCACCCGAACATCTTAT	300-400
Ph38	SUNPh10-31	(TTCT) <sub>18</sub>	AGCTGCTAGGAAATCTGAAC	TGCTTGCTCCGAATATTAAC	200-300
Ph39	SUNPh11-41	(GATG) <sub>6</sub>	AAGCGACGAAGTGAATAGAT	CTCCGATCGCTCCTCATATT	200-223
Ph40	SUNPh11-45	(TATT) <sub>6</sub>	TTGACGCCTGAGAGTTATTC	GGCGGGCATACTTTTAC	250-273
Ph41	SUNPh12-24	(CAA) <sub>8</sub>	CCCTAAGCCCTCTCTGAATA	TTAGCACCAATGTCCTACTT	214-224
Ph42	SUNPh12-45	(AGTG) <sub>7</sub>	GTTTCGAGCGTTAATGATAAG	GTGATTCGGGGTTTTTTAAG	198-207
Ph43	SUNPh12-46	(CTTTT) <sub>7</sub>	TTACACCCACATTCCTTATT	CGGACCTGGAAGAGATAAGA	304-400

### 3.2.3.5 Genetic diversity amongst *Puccinia hordei* isolates

The suitability of the microsatellite markers to determine genotypic diversity among the *Ph* individuals was evaluated. The isolates were divided into two groups (clades A, B and C; clades D and E) based on the obtained phylogram (3.2.3.6). The within-genetic diversity was determined in terms of the number of alleles, gene diversity, allelic richness, inbreeding coefficient ( $F_{IS}$  at 34 000 permutations), and observed ( $H_o$ ) and expected heterozygosity ( $H_e$ ) using FSTAT v. 2.9.3.2 (Goudet, 2001). GENCLONE v. 2.0 (Arnaud-Haond and Belkhir, 2007) was used to determine genotypic diversity and to identify distinct multi-locus genotypes (MLGs) under panmixia. To evaluate whether the observed MLGs that occurred more than once, were true clones, the  $psex$  value was calculated using MLGsim v. 2.0 (Stenberg *et al.*, 2003). Linkage disequilibrium for the clone-corrected data matrix across all loci in terms of the index of association ( $I_A$ ) and a measure corrected for the number of tested loci ( $r^2D$ ), was determined with MultiLocus v. 1.3 at 1 000 randomizations (Agapow and Burt, 2001).

### 3.2.3.6 Genetic relatedness between *Puccinia hordei* isolates

The genetic relatedness between the 48 *Ph* isolates was determined using DARwin v. 6.0.021 (Perrier *et al.*, 2003). An unweighted neighbor-joining (NJ) cluster analysis was done by constructing the phylogram based on the dissimilarity of the allelic data using 30 000 bootstraps, with the minimum proportion of valid data for each microsatellite marker set to 70%.

### 3.2.3.7 Grouping of *Puccinia hordei* isolates into sub-populations

STRUCTURE v. 2.2.4 (Pritchard *et al.*, 2000) was used to divide the 33 *Ph* MLG's into sub-populations without having any prior knowledge of their underlying genetic relationships within the South African *Ph* collection of isolates. The first step was to determine the optimal  $K$ -value from  $K = 1$  to  $K = 10$  with a 10 000 burn-in period length and 10 000 Monte Carlo Markov Chain (MCMC) repetitions after burn-in with 10 iterations per  $K$ -value. The obtained data was then used according to Evanno *et al.* (2005) using the *ad hoc*  $\Delta K$  statistic to determine the best  $K$ -value using CLUMPAK (Kopelman *et al.*, 2015). The final step was

to group the isolates into sub-populations using the optimal  $K$ -value with a 100 000 burn-in period length and 100 000 MCMC repetitions after burn-in.

Analysis of molecular variance (AMOVA; Excoffier and Lischer, 2010) was used to determine the genetic variation between the five groups (A to E) obtained from the phylogram. The analysis was conducted using Arlequin v. 3.5.2.2 (Schneider *et al.*, 2010) with the number of permutations set to 16 000. The F-statistics parameter ( $F_{ST}$ ) served as an indication of significant differentiation between the subclades when the value was greater than 0.25 (Hartl and Clark, 1997).

#### **3.2.3.8 Correlation between genotype and phenotype of *Puccinia hordei* isolates**

A seedling IT binary data matrix for the *Ph* isolates was created where avirulence was scored as 0 and virulence as 1. This was used to create a dendrogram in DARwin v. 6.0.021 as described in section (3.2.3.6). After calculating distance matrixes for both the allelic and binary data sets in GenAlEx 6.51b2 (Peakall and Smouse, 2006; 2012), the correlation between the two distance matrixes was calculated according to Mantel (1967).

## Chapter 4 Results

### 4.1 Phenotypic analysis of *Puccinia hordei* isolates

#### 4.1.1 Response of an international set of barley differentials to *Puccinia hordei* isolates

Trials were successfully carried out to determine the seedling ITs and field responses of an international set of barley differentials to South African *Ph* isolates. Variation in seedling ITs to the catalogued *Rph*-resistance genes present in the differential lines, allowed for the identification of three races (Table 4.1). The data presented in Table 4.1 represents the typical seedling ITs recorded for representative isolates of each race in replicated trials. Seedling ITs recorded for isolates of race UVPPh7235 represent that of isolates collected from 2017 to 2019 in mainly the south WC province barley production area of SA. The *Ph* isolates representative of races UVPPh3231 and UVPPh7231 typed as previously described by Van Niekerk *et al.* (2001a). The two *Ph* races UVPPh7231 and UVPPh7235 can be distinguished from race UVPPh3231 in virulence to *Rph12* with UVPPh7235 the only race with virulence to *Rph3*. The two control varieties Gus and PI 532013 were susceptible and high in their seedling IT response to all the *Ph* isolates.

The results obtained from inoculating the set of international barley differential lines (Table 4.2) at seedling stage with 77 single-pustule *Ph* isolates revealed virulence (ITs  $\geq 3$ ) among isolates to Sudan (*Rph1*), Gatam (*RphGatam*), Reka 1 (*Rph2* + *Rph19*), Gold (*Rph4*), Magnif 104 (*Rph5*), Egypt 4 (*Rph8*), Clipper BC8 (*Rph10*), Clipper BC867 (*Rph11*), Prior (*Rph19*) and Fong Tien (*Rph25*). No virulence (ITs  $\leq 2$ ) was detected for Peruvian (*Rph2*), Ricardo (*Rph2* + *Rph21*), Quinn (*Rph2* + *Rph5* + *Rph27*), Bolivia (*Rph2* + *Rph6*), Cebada Capa (*Rph7*), Abyssinian (*Rph9*), PI 531849 (*Rph13*), PI 584760 (*Rph14*), HOR 1063 (*Rph16* = *Rph15*), line 81882 (*Rph17*), line 38P18/8/1/10 (*Rph18*), Ricardo/Gus RIL (*Rph21*), line 182Q20/1/M1 (*Rph22*) and line 200A12/8/2/M2 (*Rph26*). The virulence observed for Reka 1 (*Rph2* + *Rph19*, IT = 3+) and Prior (*Rph19*, IT = 3+) was contradicted by the resistance response recorded for Peruvian (*Rph2*, IT = ;12+) to all the isolates. The variety Berg (*Rph1*, IT = 12, 3+) segregated in its response to all the *Ph* race isolates opposed to Sudan (*Rph1*, IT = 33+) that was susceptible. Seedling ITs recorded for the varieties Estate (*Rph3*) and Triumph

(*Rph12*) differentiated between *Ph* isolates of the three races. A visual representation of the seedling ITs to race UVPPh3231, UVPPh7231, and UVPPh7235 is shown in Figure 4.1.

The results from field trials only represent *Ph* race UVPPh7235. The artificial inoculations were carried out annually at the Greytown trial site with a representative isolate of this race. The presence of *Ph* race UVPPh7235 at Napier during the 2020 season was confirmed through race typing. Percentage leaf area recorded at Napier in 2020 were lower compared to the responses observed in the Greytown trials (2017 to 2019). However, similar host infection responses were recorded over seasons for the barley varieties. The field responses of the barley varieties to *Ph* were mostly in accordance with their seedling data. Exceptions included the variety Berg (*Rph1*, seedling IT = 12, 3+) that were found to be resistant (0 to 20MR) in the field as well as Egypt 4 (*Rph8*) that showed a relatively low percentage of leaf area infected over years with host infection responses that varied from MRMS to S. The host infection response for the variety Prior (*Rph19*) was also inconsistent with an RMR recorded at Napier in 2020 compared to the MSS to S responses recorded in the Greytown trials.

#### **4.1.2 Response of the Bowman lines to *Puccinia hordei* isolates**

The seedling ITs recorded for the *Rph* genes in the BW lines (Table 4.3) were mostly in accordance with their counterparts in the international differentials. These included BW682 (*Rph1*), BW746 (*Rph3*), BW752 (*Rph4*), BW755 (*Rph5*), BW758 (*Rph7*), BW759 (*Rph8*), BW683 (*Rph10*), BW684 (*Rph11*), BW761 (*Rph12*), and BW719 (*Rph15*). Contrary, discrepancies occurred for BW743 (*Rph2*) and Peruvian (*Rph2*) (Figure 4.2) which is in support of virulence recorded for *Rph2* in Reka 1 (*Rph2* + *Rph19*) in the international set. Line BW756 (*Rph6*) was high (IT = 3+) when compared with low IT (1C) recorded for *Rph6* in the line Bolivia/GUS. Similarly, the high IT (3+) for BW760 (*Rph9*) contradicted the low IT (;12+) recorded for Abyssinian (*Rph9*) in the international set (Figure 4.2). Seedling ITs recorded with UVPPh7231 and UVPPh7235 on BW686 (*Rph14*, 12+C) was slightly higher compared with that of PI 584760 (*Rph14*, ;12C) in the international set (Figure 4.3). This was more evident in the adult plant stage with PI 584760 (*Rph14*) found to be resistant (0 to 30MR) and BW686 (*Rph14*) susceptible (MSS to S) accompanied by high severities in the field trials. The MRMS (greenhouse) APR host infection response recorded for BW685

(*Rph13*) was slightly higher than the MR reaction recorded at Greytown and also higher than expected considering the low seedling ITs (;1) recorded to the *Ph* races.

**Table 4.1** Naming of *Puccinia hordei* (*Ph*) isolates into races according to their seedling infection types (ITs) recorded on the set of 12 international differential lines carrying the *Rph* genes *Rph1* to *Rph12*. Their respective added octal values are indicated in bold.

Line	<i>Rph</i> gene	ITs <sup>1</sup>	Octal value	ITs	Octal value	ITs	Octal value
Sudan	<i>Rph1</i>	33+	<b>1</b>	33+	<b>1</b>	33+	<b>1</b>
Peruvian	<i>Rph2</i>	;12+	2	;12+	2	;12+	2
Estate	<i>Rph3</i>	;1	4	;1	4	3+	<b>4</b>
Gold	<i>Rph4</i>	33-	<b>10</b>	33-	<b>10</b>	33-	<b>10</b>
Magnif 104	<i>Rph5</i>	33-	<b>20</b>	33-	<b>20</b>	33-	<b>20</b>
Bolivia	<i>Rph2+Rph6</i>	;12+	40	;12+	40	;12+	40
Cebada Capa	<i>Rph7</i>	;N	100	;N	100	;N	100
Egypt 4	<i>Rph8</i>	3C	<b>200</b>	3C	<b>200</b>	3C	<b>200</b>
Abyssinian	<i>Rph9</i>	12+	400	12+	400	12+	400
Clipper BC8	<i>Rph10</i>	3	<b>1000</b>	3	<b>1000</b>	3	<b>1000</b>
Clipper BC67	<i>Rph11</i>	3	<b>2000</b>	3	<b>2000</b>	3	<b>2000</b>
Triumph	<i>Rph12</i>	;12CN	4000	3+	<b>4000</b>	3+	<b>4000</b>
<b>Race name</b>			<b>UVPPh3231<sup>2</sup></b>		<b>UVPPh7231<sup>2</sup></b>		<b>UVPPh7235<sup>2</sup></b>

<sup>1</sup>Seedling infection types according to Park and Karakousis, (2002) with "0" = no uredinia; ";" = fleck; "1" = small uredinia with necrotic border; "2" = small to medium uredinia with necrosis/chlorosis; "3" = medium sized uredinia; "4" = large uredinia without chlorosis/necrosis; "C" = more than normal chlorosis observed; "N" = more than normal necrosis observed; "+" = larger than normal uredinia observed; "-" = smaller than normal uredinia observed; seedling infection types of 0 to 2+ were considered as low while ITs of 3 to 4 or combinations thereof were interpreted as high

<sup>2</sup>*Puccinia hordei* race names were determined by adding the octal values in bold indicating virulence (Gilmour, 1973)

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**Table 4.2** Barley differential lines, their resistance gene(s) (*Rph*), variation in low infection types (ITs) and recorded seedling ITs to the three *Puccinia hordei* (*Ph*) races identified. Field responses were recorded to race UVP<sub>h</sub>7235 in field trials planted near Greytown (KwaZulu-Natal, 2017 to 2019) and Napier (Western Cape, 2020), South Africa.

Line <sup>1</sup>	<i>Rph</i> gene(s) <sup>2</sup>	Variation in low ITs <sup>2</sup>	<i>Ph</i> races and ITs <sup>3</sup>			Greytown <sup>4</sup>			Napier <sup>4</sup>
			UVP <sub>h</sub> 3231	UVP <sub>h</sub> 7231	UVP <sub>h</sub> 7235	2017	2018	2019	2020
Gus <sup>5</sup>	Nil	Unknown	3+	3+	3+	100S	100S	90S	40S
PI 532013 <sup>6</sup>	Nil	Unknown	4	4	4	100S	100S	100S	100S
Sudan <sup>5</sup>	<i>Rph1</i>	1, 2C	33+	33+	33+	50MS	40MS	60MS	20MS
Berg <sup>5</sup>	<i>Rph1</i>	0N, 1++	12, 3+	12, 3+	12, 3+	20MR	5MR	0	0
Peruvian <sup>5</sup>	<i>Rph2</i>	1, 1++C	;12+	;12+	;12+	20MR	10MRR	5MRR	0
Gatam <sup>5</sup>	<i>RphGatam</i>	;1, 1-	3+	3+	3+	60MSS	100S	50MSS	30S
Reka 1 <sup>5</sup>	<i>Rph2+Rph19</i>	0;, 2C	3+	3+	3+	50MSS	100S	60MSS	5S
Ricardo <sup>5</sup>	<i>Rph2+Rph21</i>	0;, 12-C	12C	12C	12C	10MR	10MRR	0	0
Estate <sup>5</sup>	<i>Rph3</i>	0;, 1=CN	;1	;1	3+	80S	100S	60S	30S
Gold <sup>5</sup>	<i>Rph4</i>	1, 2+C	33-	33-	33-	40MS	60MS	40MS	30S
Quinn <sup>5</sup>	<i>Rph2+Rph5+Rph27</i>	0;, 1+N	;12	;12	;12	10MR	5MRR	0	TMR
Magnif 104 <sup>5</sup>	<i>Rph5</i>	0;, 1N	33-	33-	33-	40MS	40MS	40MS	15S
Bolivia <sup>5</sup>	<i>Rph2+Rph6</i>	1, 1++	;12+	;12+	;12+	0	0	0	0
Bolivia/Gus RIL <sup>5</sup>	<i>Rph6</i>	2C	1C	1C	1C	Nt <sup>8</sup>	Nt	Nt	0
Cebada Capa <sup>5</sup>	<i>Rph7</i>	0;, ;CN	;N	;N	;N	5MR	0	0	0
Egypt 4 <sup>5</sup>	<i>Rph8</i>	2=C, 3=C	3C	3C	3C	20MRMS	20MRMS	20MS	10S
Abyssinian <sup>5</sup>	<i>Rph9</i>	;1, 1-C	;12+	;12+	;12+	5MR	0	0	0
Clipper BC8 <sup>5</sup>	<i>Rph10</i>	1+, 1++	3	3	3	40MSS	60S	70MSS	40S
Clipper BC867 <sup>5</sup>	<i>Rph11</i>	1, 1++	3	3	3	60MSS	60S	70MSS	25S
Triumph <sup>5</sup>	<i>Rph12</i>	0;, 1+CN	12CN	3+	3+	60MSS	50S	70S	30S
PI 531849 <sup>5</sup>	<i>Rph13</i>	0, 1CN	1CN	1CN	1CN	10MR	5RMR	0	TRMR
PI 584760 <sup>5</sup>	<i>Rph14</i>	1CN, 1++	;12C	;12C	;12C	30MR	20MR	20MRR	0
HOR 1063 <sup>5</sup>	<i>Rph16 (Rph15)</i>	0;N	;1	;1	;1	Nt	Nt	Nt	0
81882 <sup>7</sup>	<i>Rph17</i>	12C	1	1	1C	Nt	Nt	Nt	Nt

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**Table 4.2 (cont.)** Barley differential lines, their resistance gene(s) (*Rph*), variation in low infection types (ITs) and recorded seedling ITs to the three *Puccinia hordei* (*Ph*) races identified. Field responses were recorded to race UVPPh7235 in field trials planted near Greytown (KwaZulu-Natal, 2017 to 2019) and Napier (Western Cape, 2020), South Africa.

Line <sup>1</sup>	<i>Rph</i> gene(s) <sup>2</sup>	Variation in low ITs <sup>2</sup>	<i>Ph</i> races and ITs <sup>3</sup>			Greytown <sup>4</sup>			Napier <sup>4</sup>
			UVPPh3231	UVPPh7231	UVPPh7235	2017	2018	2019	2020
38P18/8/1/10 <sup>7</sup>	<i>Rph18</i>	0;	0;	0;	0;	Nt	Nt	Nt	Nt
Prior (P) <sup>5</sup>	<i>Rph19</i>	1, 2C	3+	3+	3+	60MSS	60S	70MSS	10RMR
Ricardo/Gus RIL <sup>5</sup>	<i>Rph21</i>	12+C	1C	1+C	1+C	Nt	Nt	Nt	TR
182Q20/1/M1 <sup>7</sup>	<i>Rph22</i>	;C	;1	;1	;1	Nt	Nt	Nt	Nt
Fong Tien <sup>7</sup>	<i>Rph25</i>	33+	3C	3C	3C	Nt	Nt	Nt	20S
200A12/8/2/M2 <sup>7</sup>	<i>Rph26</i>	2-C	1C	1C	1+C	Nt	Nt	Nt	Nt

<sup>1</sup>Differential lines and *Rph* genes according to Ivandic *et al.* (1998); Pickering *et al.* (1998); Pickering *et al.* (2000); Sandhu *et al.* (2012); Johnston *et al.* (2013); Elmansour *et al.* (2017); Kavanagh *et al.* (2017)

<sup>2</sup>*Rph5/Rph6* (Zhong *et al.*, 2003), *Rph9/Rph12* (Borovkova *et al.*, 1998) and *Rph15/Rph16* (Weerasena *et al.*, 2004) were reported to be allelic. *Rph15/Rph16* are now reported as the same gene (Chen *et al.*, 2020); Low ITs as in Park *et al.* (2015); Kavanagh *et al.* (2017); Martin (2018); Yu *et al.* (2018)

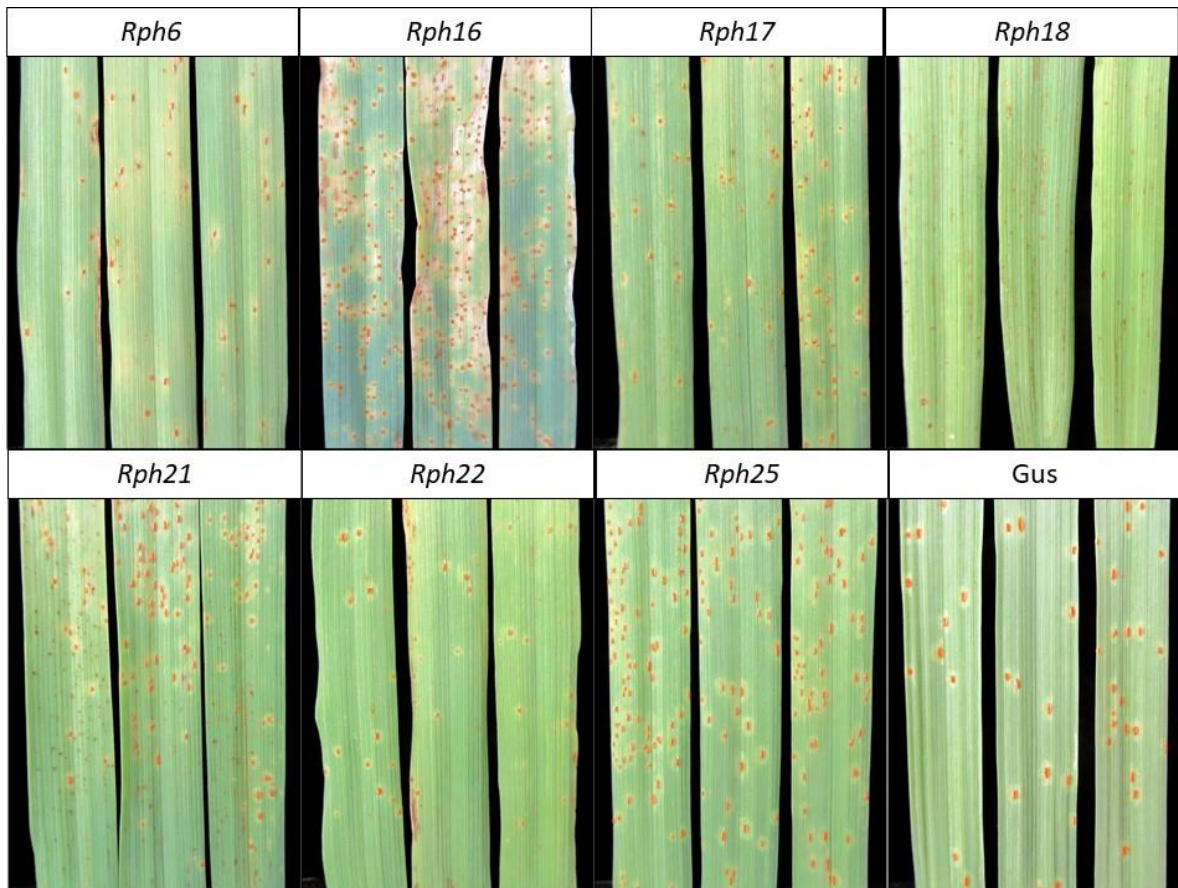
<sup>3</sup>Infection types according to Park and Karakousis (2002) with "0" = no uredinia; ";" = fleck; "1" = small uredinia with necrotic border; "2" = small to medium uredinia with necrosis/chlorosis; "3" = medium sized uredinia; "4" = large uredinia without chlorosis/necrosis. The letter "C" = more than normal chlorosis; "N" = more than normal necrosis; "+" = larger than normal uredinia and "-" = smaller than normal uredinia, seedling infection types of 0 to 2+ were characterised as low while ITs of 3 to 4 or combinations thereof were interpreted as high

<sup>4</sup>The presence of *Ph* race UVPPh7235 was confirmed with race typing at all field localities and years. Data represents the highest field response values for percentage leaf area infected (0 – 100%, Modified Cobb Scale Peterson *et al.*, 1948) and host infection response (R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible; TR = trace resistance; Roelfs *et al.*, 1992) recorded for each entry at the end of each season

<sup>5</sup>Seed provided by Prof Robert Park (Plant Breeding Institute, The University of Sydney, Australia); <sup>6</sup>Prof Brian Steffenson (Department of Plant Pathology, University of Minnesota, St Paul, MN 55108, United States of America) and <sup>7</sup>Dr Paul Johnson (Plant and Food Research, Lincoln, New Zealand)

<sup>8</sup>Not tested due to seed availability

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**Figure 4.1** Seedling infection types (ITs) recorded for the international differential lines Bolivia/Gus (*Rph6*, typical IT = 1C), HOR 1063 (*Rph16* = *Rph15*, ;1), 81882 (*Rph17*, 1 to 1C), 38P18/8/1/10 (*Rph18*, 0;), Ricardo/Gus (*Rph21*, 1C to 1+C], 182Q20/1/M1 (*Rph22*, ;1) and Fong Tien (*Rph25*, 3C). The variety Gus (nil, 3+) was the susceptible control. Photos were taken 12 days after inoculation with each plate representing from left to right infected seedling leaves for *Puccinia hordei* races UVP<sub>h</sub>3231, UVP<sub>h</sub>7231 and UVP<sub>h</sub>7235.

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**Table 4.3** Seedling infection types (ITs) recorded to *Puccinia hordei* (*Ph*) races on the Bowman (BW) leaf rust introgression lines and their field and greenhouse responses to UVPPh7235 in trials planted near Greytown (KwaZulu-Natal, 2019) and Napier (Western Cape, 2020), South Africa.

BW line <sup>1,2</sup>	<i>Rph</i> gene	<i>Rph</i> allele	Reported low ITs	UVPPh races and ITs <sup>3</sup>			Greytown <sup>4</sup>	Napier <sup>4</sup>	Adult plant responses recorded for <i>Ph</i> races in the greenhouse		
				Ph3231	Ph7231	Ph7235	2019	2020	UVPPh3231	UVPPh7231	UVPPh7235
BW682	<i>Rph1</i>	<i>Rph1.a</i>	0;	3+	3+	3+	60S	100S	S	S	S
BW743	<i>Rph2</i>	<i>Rph2.t</i>	1, 2	3+	3+	3+	60S	100S	S	S	S
BW746	<i>Rph3</i>	<i>Rph3.c</i>	0, 2	;1CN	;1CN	3+	70S	90S	R	R	S
BW752	<i>Rph4</i>	<i>Rph4.d</i>	1, 2	3+	3+	3+	70S	90S	S	S	S
BW755	<i>Rph5</i>	<i>Rph5.e</i> <sup>5</sup>	1, 2	3+	3	3	70S	90S	S	S	S
BW756	<i>Rph6</i>	<i>Rph5.f</i> <sup>5</sup>	1	3+	3+	3+	70S	100S	S	S	S
BW758	<i>Rph7</i>	<i>Rph7.g</i>	0, 2	;N	;1-N	;1-N	20MR	TR	R	RMR	RMR
BW759	<i>Rph8</i>	<i>Rph8.h</i>	0;, 2	3C	3C	3C	70S	50S	S	S	MSS
BW760	<i>Rph9</i>	<i>Rph9.i</i>	1, 2	3+	3+	3+	70S	90S	MS	S	S
BW683	<i>Rph10</i>	<i>Rph10.o</i>	1, 2	3	3	3	50S	90S	S	S	S
BW684	<i>Rph11</i>	<i>Rph11.p</i>	2	3	3	3	50S	80S	S	S	S
BW761	<i>Rph12</i>	<i>Rph9.z</i>	1	12CN	3+	3+	70S	90S	R	S	S
BW685	<i>Rph13</i>	<i>Rph13.x</i>	0;, 2	;1	;1	;1	20MR	Nt <sup>6</sup>	MRMS	MRMS	MRMS
BW686	<i>Rph14</i>	<i>Rph14.ab</i>	0;, 2	12C	12+C	12+C	70S	70S	MSS	S	S
BW719	<i>Rph15</i>	<i>Rph15.ad</i>	0;, 2	;CN	;CN	;CN	20MRR	TR	R	R	R
PI 532013	Nil			4	4	4	100S	100S	S	S	S

<sup>1</sup>Bowman lines, *Rph* genes, alleles, and low infection type responses as reported in Martin (2018)

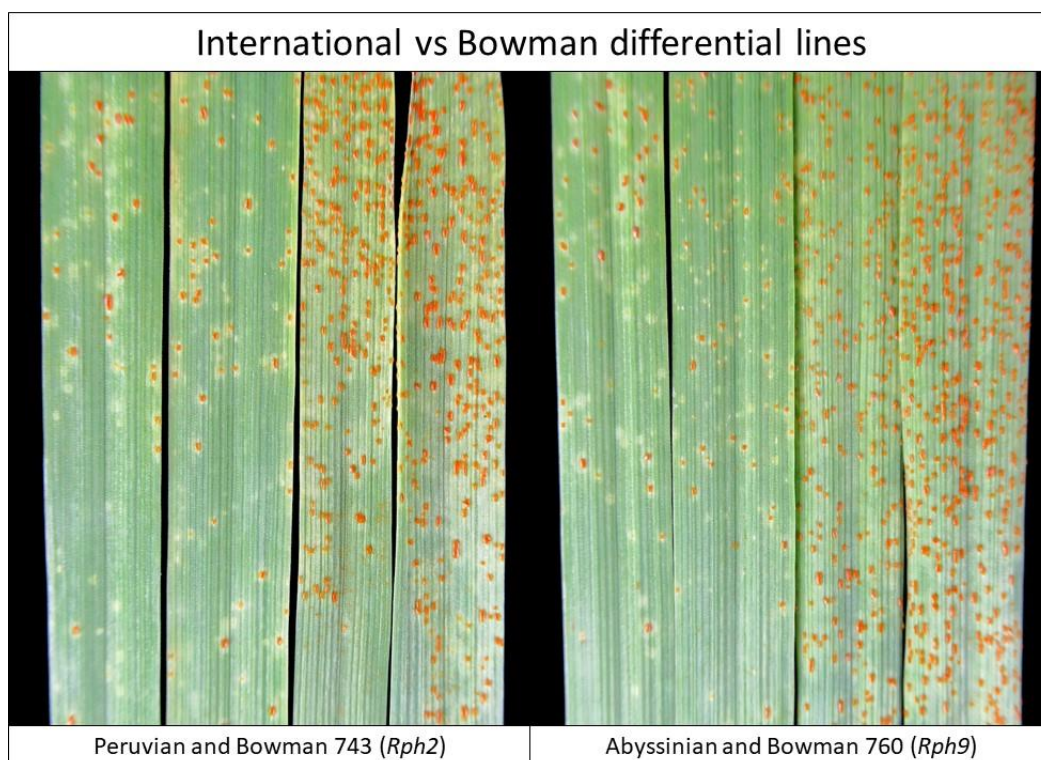
<sup>2</sup>Seed provided by Prof Brian Steffenson, Department of Plant Pathology, University of Minnesota, St Paul, MN 55108, United States of America

<sup>3</sup>Infection types according to Park and Karakousis (2002) with “0” = no uredinia; “;” = fleck; “1” = small uredinia with necrotic border; “2” = small to medium uredinia with necrosis/chlorosis; “3” = medium sized uredinia; “4” = large uredinia without chlorosis/necrosis. The letter “C” = more than normal chlorosis; “N” = more than normal necrosis; “+” = larger than normal uredinia and “-” = smaller than normal uredinia. Infection types of 3 or higher were considered to indicate compatibility (i.e. virulence/susceptible host)

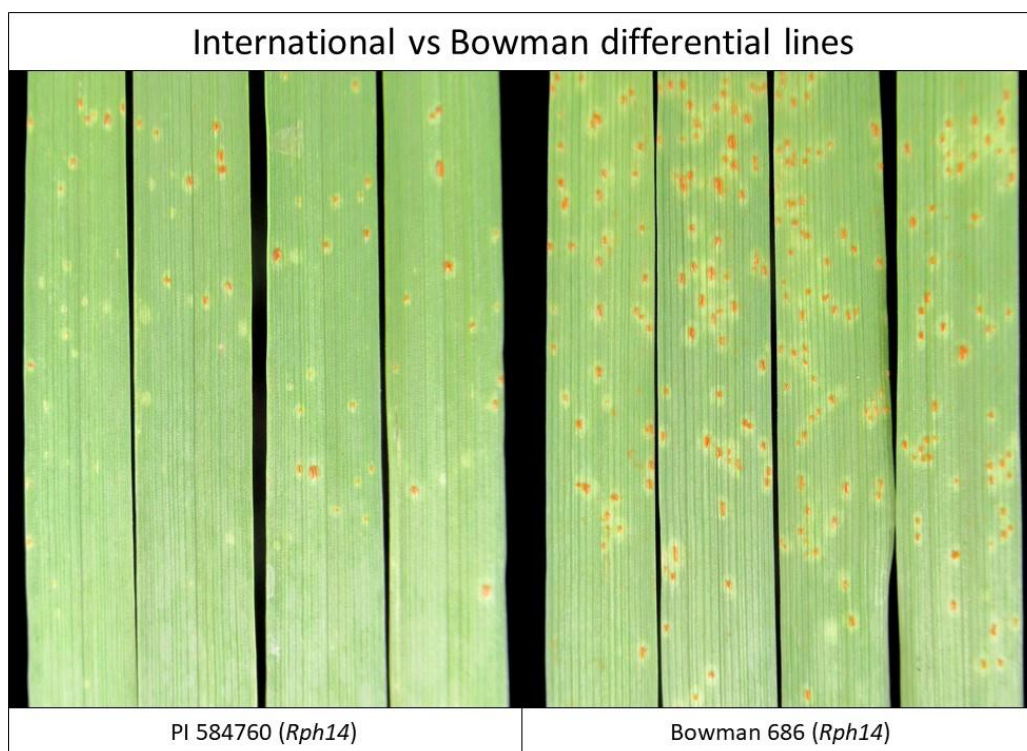
<sup>4</sup>The presence of *Ph* race UVPPh7235 was confirmed with race typing at all field localities and years. Data represents the highest field response values for percentage leaf area infected (0 – 100%, Modified Cobb Scale Peterson *et al.*, 1948) and host infection response (R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible; TR= trace resistance; Roelfs *et al.*, 1992) recorded for each entry at the end of each season

<sup>5</sup>*Rph5/Rph6* allelic (Zhong *et al.*, 2003)

<sup>6</sup>Not tested due to seed availability



**Figure 4.2** Comparative seedling infection types recorded for *Rph2* in Peruvian (;12+) and BW743 (3+) on the left and on the right *Rph9* in Abyssinian (;12+) and BW760 (3+). Photos were taken 12 days after inoculation with *Puccinia hordei* race UVPPh7235.



**Figure 4.3** Seedling infection types recorded for *Rph14* in line PI 584760 (;12C) and BW686 (12+C). Photos were taken 12 days after inoculation with *Puccinia hordei* race UVPPh7235.

#### **4.1.3 Response of barley varieties with adult plant resistance genes to *Puccinia hordei* isolates**

The seedling ITs and adult plant field and greenhouse responses recorded for four barley varieties with designated APR genes and two susceptible control varieties to three South African *Ph* races are summarised in Table 4.4. The seedling data revealed high ITs for Yerong (*Rph2* + *Rph23*), Flagship (*Rph20*) and the two controls Gus and PI 532013. The variety Lenka (*Rph20* + *Rph23* + *Rph24*) showed a low IT (;N) to *Ph* races UVPPh3231 and UVPPh7231 and a high IT (3+) to race UVPPh7235. Baronesse showed low (;12) ITs to the three *Ph* races.

Similar adult plant infection responses were recorded for PI 532013 (S) and Flagship (MS) under field and greenhouse conditions. Field responses recorded for Yerong to *Ph* race UVPPh7235 varied from 5MS to 60MS with more resistant (MRR) infection responses recorded under greenhouse conditions to isolates of the three *Ph* races (Figure 4.4). In accordance with the seedling IT data, Lenka was R to *Ph* races UVPPh3231 and UVPPh7231 and MS to race UVPPh7235. The field infection responses for Lenka over three seasons to *Ph* race UVPPh7235 varied from 30S to 60S with a MS response observed under greenhouse conditions. Similarly, the infection responses recorded for Gus (field = S, greenhouse = MS) and Baronesse (field= MR, greenhouse= RMR) varied slightly when their field and greenhouse data was compared. Percentage leaf area infected in the field trials was consistently 100% for PI 532013, while for Gus it was only 40% at Napier and consistent at Greytown but lower at Napier.

#### **4.1.4 Response of South African barley varieties to *Puccinia hordei* isolates**

The seedling ITs and adult plant field and greenhouse responses recorded for 10 barley varieties planted in SA, as well as two susceptible control varieties, to isolates representative of three *Ph* races are shown in Table 4.5. The seedling data for the three races revealed high ITs for the susceptible controls PI 532013 and Gus, whereas the varieties Agulhas and Cristalia (Figure 4.5) had low ITs. Overture (Figure 4.5), Deveron, Genie, Kadie, KWS Irina and Elim (Figure 4.6) displayed low seedling ITs to *Ph* races

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UVPPh3231 and UVPPh7231 and high ITs to UVPPh7235. The varieties Erica and Hessekwa (Figure 4.6) showed low seedling ITs to *Ph* race UVPPh3231 and high ITs towards races UVPPh7231 and UVPPh7235. The adult plant responses determined under field and greenhouse conditions were in accordance with the seedling ITs for the three *Ph* races. However, the variety Agulhas consistently showed a higher percentage leaf area infected (5 to 60%) accompanied by a more compatible infection response (MRMS) in field trials compared with Cristalia (T = trace to 10%, MRR). Except for the varieties Genie (80S) (Figure 4.7) and Overture (80S) that showed increased susceptibility at Napier (2020) compared to Greytown (2017 to 2019), the field data for individual varieties corresponded well over seasons and localities. The greenhouse infection response data was mostly in accordance with the field data. However, slightly more resistant host responses were observed for Agulhas (field = MRMS, greenhouse = MR), Deveron (MS to MRMS), Elim (MS to MRMS), Hessekwa (MS to MRMS) (Figure 4.7) and Gus (S to MS) under greenhouse conditions.

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**Table 4.4** Seedling infection types (ITs) and adult plant field and greenhouse responses recorded for barley varieties with designated adult plant resistance genes to *Puccinia hordei* (*Ph*). Field trials were planted near Greytown (KwaZulu-Natal, 2018 to 2019) and Napier (Western Cape, 2020), South Africa.

Variety <sup>1,2</sup>	<i>Rph</i> gene	Seedling ITs <sup>3</sup>			Greytown <sup>4</sup>			Napier <sup>4</sup>			Greenhouse adult plant infection response		
		UVPh3231	UVPh7231	UVPh7235	2018	2019	2020	UVPh3231	UVPh7231	UVPh7235			
Yerong	<i>Rph2+Rph23</i>	3	3	3+	30MS	60MS	5MS	MRR	MRR	MRR			
Lenka	<i>Rph20+Rph23+Rph24</i>	;N	;N	3+	60S	60S	30S	R	R	MS			
Baronesse	<i>Rph20+Rph24</i>	;12CX	;12	;12	5MR	0	0	MRR	MRR	MRR			
Flagship	<i>Rph20</i>	3-	3-	3	30MS	50MS	10S	MS	MS	MS			
Gus	Nil	3+	3+	3+	100S	90S	40S	MSS	MSS	MSS			
PI 532013	Nil	4	4	4	100S	100S	100S	S	S	S			

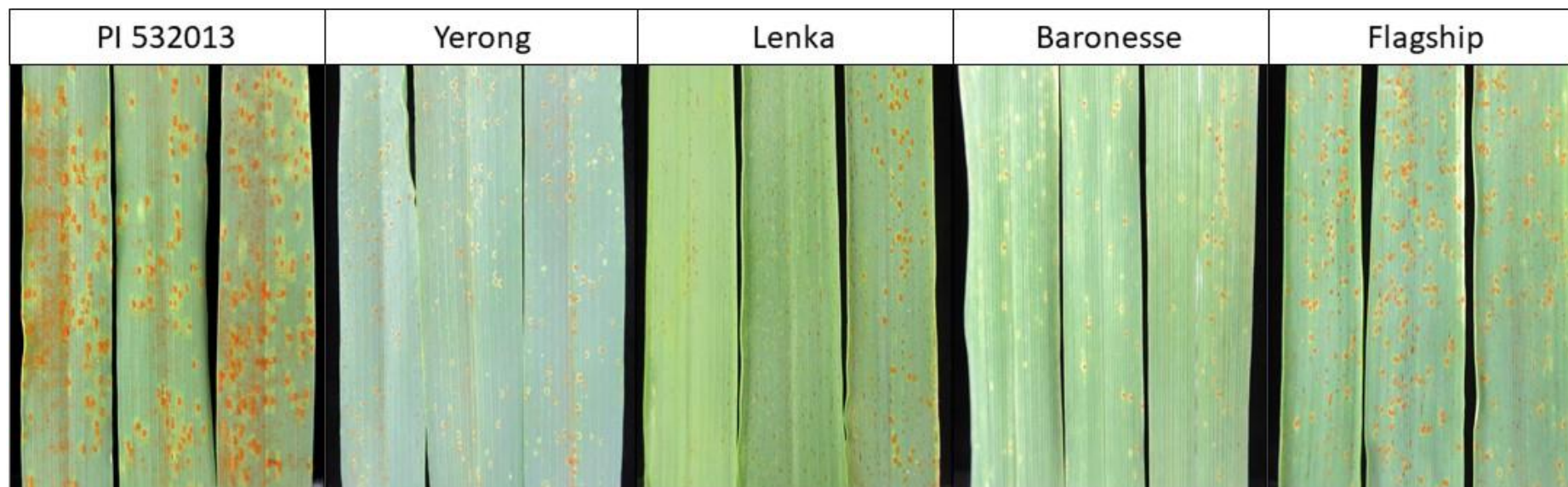
<sup>1</sup>Seed of barley varieties was provided by Mr Daniel de Klerk (ABInBev, Caledon, South Africa) and Dr Davinder Singh (Plant Breeding Institute, The University of Sydney, Australia)

<sup>2</sup>*Rph* genes as reported in Borovkova *et al.* (1997), Hickey *et al.* (2011), Singh *et al.* (2015), and Ziems *et al.* (2017)

<sup>3</sup>Infection types according to Park and Karakousis (2002) with “;” = fleck; “1” = small uredinia with necrotic border; “2” = small to medium uredinia with necrosis/chlorosis; “3” = medium sized uredinia; “4” = large uredinia without chlorosis/necrosis. The letter “N” = more than normal necrosis; “C” = more than normal chlorosis observed; “X” = random distribution of variable sized uredinia; “+” = larger than normal uredinia and “-” = smaller than normal uredinia. Infection types of 3 or higher were considered to indicate compatibility (i.e. virulence/susceptible host)

<sup>4</sup>The presence of *Ph* race UVPh7235 was confirmed with race typing at all field localities and years. Data represents the highest field response values for percentage leaf area infected (0 – 100%, Modified Cobb Scale Peterson *et al.*, 1948) and host infection response (R = resistant; MR = moderately resistant; MS = moderately susceptible and S = susceptible; Roelfs *et al.*, 1992) recorded for each entry at the end of each season. Greenhouse adult plant infection responses were recorded 15 days post-inoculation

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**Figure 4.4** Flag leaf infection responses of barley varieties with designated adult plant resistance genes (from left to right) PI 532013 (susceptible control, S), Yerong (*Rph2* + *Rph23*, MRR), Lenka (*Rph20* + *Rph23* + *Rph24*, R to MS), Baronesse (*Rph20* + *Rph24*, MRR) and Flagship (*Rph20*, MS). Photos were taken 15 days after inoculation with each plate representing from left to right *Puccinia hordei* races UVPh3231, UVPh7231 and UVPh7235.

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**Table 4.5** Seedling infection types (ITs) as well as adult plant field and greenhouse responses of South African barley varieties and susceptible controls to *Puccinia hordei* (*Ph*) races. Field trials were planted near Greytown (KwaZulu-Natal, 2017 to 2019) and Napier (Western Cape, 2020).

Variety <sup>1</sup>	Seedling ITs <sup>2</sup>			Greytown <sup>3</sup>			Napier <sup>3</sup>	Greenhouse adult plant infection response <sup>4</sup>		
	UVPPh3231	UVPPh7231	UVPPh7235	2017	2018	2019	2020	UVPPh3231	UVPPh7231	UVPPh7235
Agulhas	;12	;12	;12	60MRMS	20MRMS	10MRMS	5MS	MR	MR	MR
Cristalia	12C	12C	12C	Nt <sup>5</sup>	10MR	5MRR	TMR	RMR	RMR	RMR
Deveron	;12+	;12+	3C	10MS	20MS	50MS	Nt <sup>5</sup>	RMR	MR	MRMS
Elim	;N	;1N	3+C	50MS	20MS	40MS	TMS	R	R	MRMS
Erica	12C	3C	3+C	80MS	40MSS	40MS	10S	RMR	MS	MS
Genie	;N	;1N	3CX	15MS	20MS	30MSS	80S	R	R	MSS
Hessekwa	12C	3X+	3X+	40MS	20MS	20MS	10MS	RMR	MRMS	MRMS
Kadie	;1N	;1N	3+C	20MS	20MS	50MS	40MS	R	R	MS
KWS Irina	;N	;1N	3+	5MS	20MS	50MS	Nt <sup>5</sup>	RMR	RMR	MS
Overture	;N	;1N	3C	20MS	10MS	30MS	80S	R	R	MS
Gus	3+	3+	3+	100S	100S	90S	40S	MS	MS	MS
PI 532013	4	4	4	100S	100S	100S	100S	S	S	S

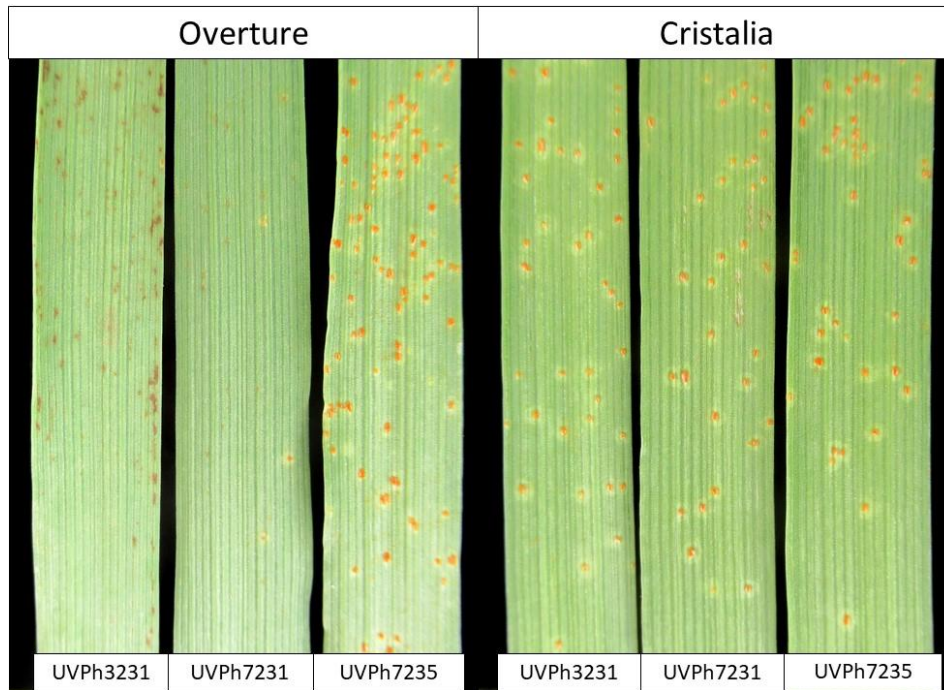
<sup>1</sup>Seed of barley varieties was provided by Mr Daniel de Klerk (ABInBev, Caledon, South Africa)

<sup>2</sup>Infection types according to Park and Karakousis (2002) with “;” = fleck; “1” = small uredinia with necrotic border; “2” = small to medium uredinia with necrosis/chlorosis; “3” = medium sized uredinia; “4” = large uredinia without chlorosis/necrosis. The letter “C” = more than normal chlorosis; “N” = more than normal necrosis; “X” = random distribution of variable sized uredinia; “+” = larger than normal uredinia. Infection types of 3 or higher were considered to indicate compatibility (i.e. virulence/susceptible host)

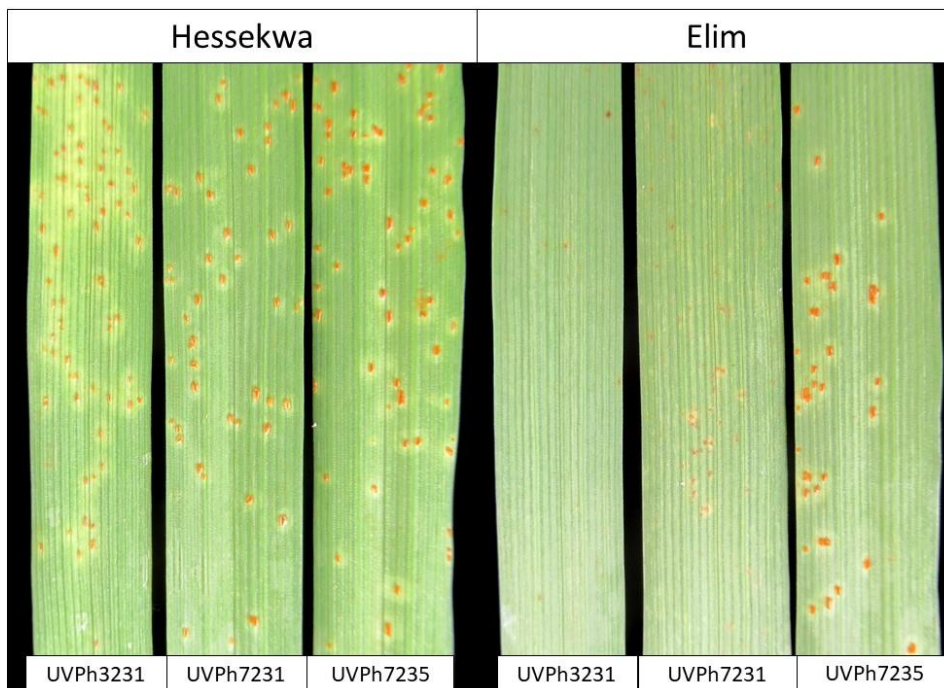
<sup>3</sup>The presence of *Ph* race UVPPh7235 was confirmed with race typing at all field localities and years. Data represents the highest field response values for percentage leaf area infected (0 – 100%, Modified Cobb Scale Peterson *et al.*, 1948) and host infection response (R = resistant; MR = moderately resistant; MS = moderately susceptible; S = susceptible; T = trace; Roelfs *et al.*, 1992) recorded for each entry at the end of each season

<sup>4</sup>Data presented include the highest plant infection response over two trial replicates for each entry

<sup>5</sup>Not tested due to seed availability

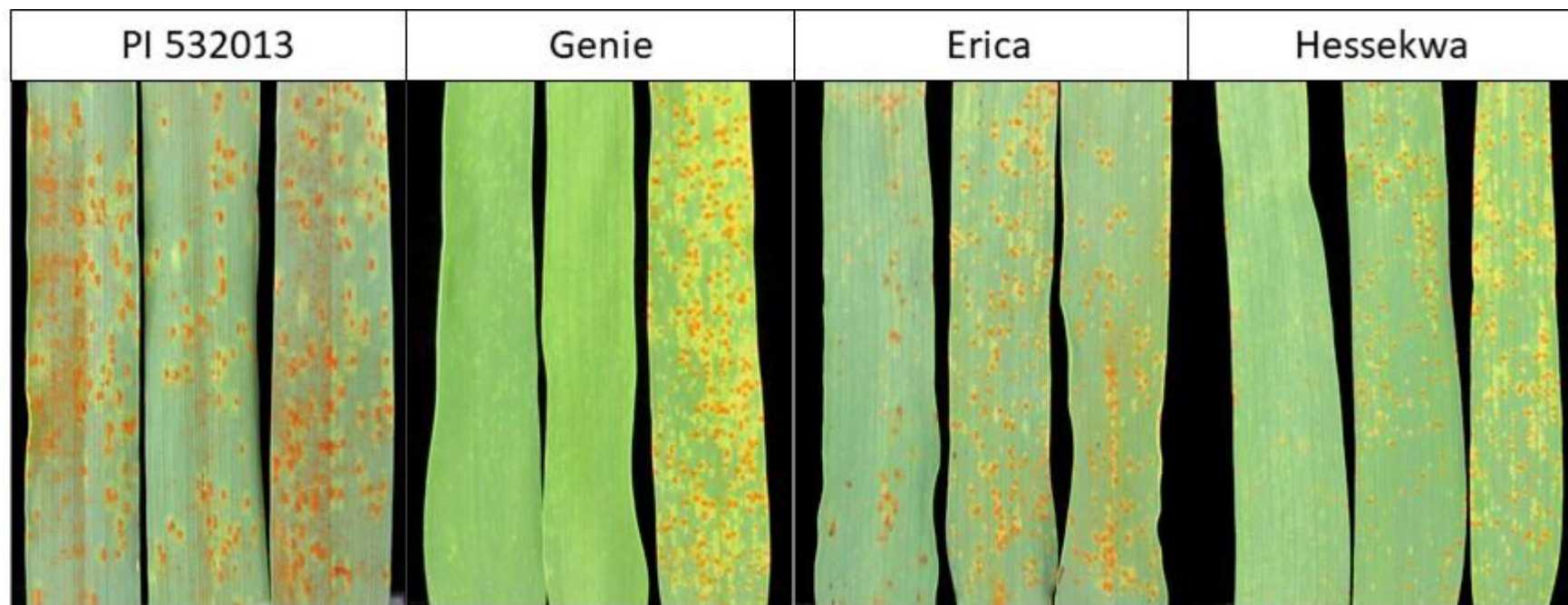


**Figure 4.5** Seedling infection types for the barley varieties Overture and Cristalia to *Puccinia hordei* races (left to right) UVPh3231, UVPh7231, and UVPh7235 12 days after inoculation.



**Figure 4.6** Seedling infection types for the barley varieties Hessekwa and Elim to *Puccinia hordei* races (left to right) UVPh3231, UVPh7231 and UVPh7235 12 days after inoculation.

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**Figure 4.7** Flag leaf infection responses of barley varieties 15 days post-inoculation under greenhouse conditions. Each plate represents from left to right *Puccinia hordei* races UVPh3231, UVPh7231 and UVPh7235.

## 4.2 Genotypic analysis of the South African *Puccinia hordei* isolates

### 4.2.1 Genetic diversity amongst the South African *Puccinia hordei* isolates

Within-genetic diversity of the 48 *Ph* isolates genotyped with the 20 microsatellite primers, is indicated in Table 4.6. The number of alleles ranged from one to four with an average of 2.55 alleles per marker. The low gene diversity and allelic richness levels (average 0.288 and 0.287 respectively), indicated low genetic diversity amongst the isolates. The inbreeding coefficient ( $F_{IS}$ ) had a negative average of -0.325 amongst all individuals. In terms of heterozygosity, the individuals had a higher  $H_O$  (0.505) than  $H_E$  (0.286) value. The values for index of association ( $I_A$ ) and a measure corrected for the number of tested loci ( $r^2_D$ ), were 0.15 and 0.02 respectively, which differed significantly from 0 ( $P < 0.001$ ). Together, the linkage disequilibrium values, as well as the  $H_O$  and highly significant  $p_{sex}$  values ( $P < 0.001$ ) suggested that the South African *Ph* population is clonal, while the strongly negative  $F_{IS}$  is an indication of excessive heterozygosity.

The ability of the 20 microsatellite markers to discriminate between each of the 48 *Ph* isolates was tested with the detection of MLGs (Table 4.7). A total of 33 MLGs were identified with Ph1.2\_Np2018 and Ph7.1\_Cl2019; Ph11.1\_Rs2018 and Ph11.2\_Rs2018; Ph8.1\_Kp2019 and Ph4.1\_Np2018; Ph2\_Rs2017, Ph7\_Rs2017 and Ph6\_Rs2017; Ph14.1\_Rs2018, Ph7.1\_Np2018, Ph16.1\_Rs2018, Ph2.1\_Np2018, Ph1\_Rs2017, Ph16.1\_Np2019, Ph16.2\_Rs2018, Ph3.1\_Np2018, Ph4\_Rs2017, Ph10.1\_Cl2019 and Ph1.1\_Np2019 all being clonal.

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**Table 4.6** Average genetic diversity amongst 48 *Puccinia hordei* (*Ph*) isolates using 20 microsatellite markers based on the average number of alleles, gene diversity, allelic richness, inbreeding coefficient ( $F_{IS}$ ) and observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity.

Marker name	Number of alleles	Gene diversity	Allelic richness	$F_{IS}$	Heterozygosity/locus	
					$H_O$	$H_E$
SUNPh7-36	2	0.021	0.021	0.000	0.021	0.021
SUNPh1-42	2	0.500	0.500	-1.000	1.000	0.500
SUNPh7-31	1	0.000	0.000	0.000	0.000	0.000
SUNPh7-38	2	0.042	0.042	1.000	0.000	0.042
SUNPh8-30	2	0.061	0.061	-0.022	0.063	0.061
SUNPh10-28	2	0.500	0.500	-1.000	1.000	0.500
SUNPh10-16	3	0.511	0.511	-0.958	1.000	0.511
SUNPh10-31	1	0.000	0.000	0.000	0.000	0.000
SUNPh10-30	1	0.000	0.000	0.000	0.000	0.000
SUNPh11-41	1	0.000	0.000	0.000	0.000	0.000
SUNPh11-45	3	0.510	0.510	-0.919	0.979	0.510
SUNPh10-44	4	0.580	0.580	-0.651	0.957	0.580
SUNPh12-24	1	0.000	0.000	0.000	0.000	0.000
SUNPh1-28	4	0.523	0.523	-0.913	1.000	0.523
SUNPh9-07	4	0.581	0.581	-0.683	0.978	0.581
SUNPh12-45	2	0.500	0.500	-1.000	1.000	0.500
SUNPh12-46	4	0.546	0.546	-0.830	1.000	0.546
SUNPh9-37	4	0.124	0.124	0.658	0.043	0.124
SUNPh9-46	4	0.546	0.531	-0.883	1.000	0.531
SUNPh10-42	4	0.206	0.214	0.708	0.060	0.192
<b>Average/locus</b>	<b>2.550</b>	<b>0.288</b>	<b>0.287</b>			
Average $F_{IS}$ /population				<b>-0.325</b>		
Average heterozygosity					<b>0.505</b>	<b>0.286</b>

**Table 4.7** List of the 33 multi-locus genotypes (MLGs) derived from the 48 *Puccinia hordei* (*Ph*) isolates and their clonal MLGs.

Number	MLGs	Clonal MLGs		
1	Ph17.1_Np2019			
2	Ph13.1_Np2019			
3	Ph15.1_Rs2018			
4	Ph6.1_Cl2019			
5	Ph5.1_Np2018			
6	Ph5_Rs2017			
7	Ph10.1_Np2018			
8	Ph8.1_Np2018			
9	Ph5.1_HA2019			
10	Ph3.1_Np2019			
11	Ph4.1_HA2019			
12	Ph1.2_Np2018	Ph7.1_Cl2019		
13	Ph11.1_Rs2018	Ph11.2_Rs2018		
14	Ph14.1_Rs2018	Ph7.1_Np2018	Ph16.1_Rs2018	Ph2.1_Np2018
		Ph1_Rs2017	Ph16.1_Np2019	Ph16.2_Rs2018
		Ph3.1_Np2018	Ph4_Rs2017	Ph10.1_Cl2019
		Ph1.1_Np2019		
15	Ph13.1_Rs2018			
16	Ph2.1_Np2019			
17	Ph14.1_Np2019			
18	Ph11.1_Rs2019			
19	UVPh7231			
20	Ph4.2_Np2018			
21	Ph9.1_Np2018			
22	UVPh3231			
23	Ph12.1_Np2019			
24	Ph3_Rs2017			
25	Ph5.2_Np2018			
26	UVPh7235			
27	Ph8.1_Kp2019	Ph4.1_Np2018		
28	Ph9.1_Vk2019			
29	Ph6.1_Np2018			
30	Ph15.1_Np2019			
31	Ph2_Rs2017	Ph7_Rs2017	Ph6_Rs2017	
32	Ph5.2_HA2019			
33	Ph12.1_Rs2018			

#### 4.2.2 Genetic comparison of South African *Puccinia hordei* isolates

The genetic relatedness of all 48 *Ph* isolates was determined with a prepared phylogram based on the allelic data matrix (Figure 4.8). The reference isolates for races UVPPh3231 and UVPPh7231 date back to *Ph* collections made during 1994 and 1998, respectively. Isolate Ph3\_Gt2015 was selected to represent *Ph* race UVPPh7235, as this was the first isolate detected in SA that typed to this race.

Overall bootstrap support for the different clades and sub-clades was low with only two branches having support above 70% (Ph5.1\_HA2019 and Ph8.1\_Np2018; Ph17.1\_Np2019 and Ph12.1\_CI2018). The phylogram showed little genetic variation amongst all races and field isolates irrespective of the time and locality of collection. The three isolates representing races UVPPh3231, UVPPh7231 and UVPPh7235 respectively, clustered in different clades showing some genetic variation. However, the *Ph* field isolates that were all typed as race UVPPh7235, were represented in all clades, even clustering very close to races UVPPh3231 and UVPPh7231.

The unrooted phylogram for all *Ph* isolates consisted of five clades (Figure 4.8). Clade A included race UVPPh7235, with virulence to *Rph3* and *Rph12*, and 10 field isolates from different localities and years of collection, which were all subsequently described as race UVPPh7235. These isolates included Ph13.1\_Rs2018, Ph11.1\_Rs2018, Ph11.2\_Rs2018, Ph6.1\_CI2019, Ph13.1\_Np2019, Ph9.1\_Np2018, Np5.1\_HA2019, Ph5.2\_HA2019, Ph8.1\_Np2018 and Ph3.1\_Np2019. All these isolates shared at least 90% genetic similarity.

Clade B included race UVPPh3231 with avirulence to *Rph3* and *Rph12*, as well as six field isolates from different localities and years that typed as race UVPPh7235. The included isolates were Ph5.1\_Np2018, Ph5.2\_Np2018, Ph5\_Rs2017, Ph11.1\_Rs2019, Ph6.1\_Np2018 and Ph2.1\_Np2019. All these isolates shared 95% genetic similarity.

Clade C consisted of seven field isolates that typed as race UVPPh7235, namely Ph4.1\_HA2019, Ph3\_Rs2017, Ph12.1\_Np2019, Ph14.1\_Np2019, Ph4.2\_Np2018, Ph15.1\_Np2019 and Ph15.1\_Rs2018. These isolates shared 95% genetic similarity amongst themselves.

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Clade D showed 100% genetic similarity amongst the eight field isolates Ph2.1\_Np2018, Ph10.1\_Cl2019, Ph1\_Rs2017, Ph16.1\_Rs2018, Ph1.1\_Np2019, Ph14.1\_Rs2018, Ph7.1\_Np2018 and Ph4\_Rs2017. All isolates typed as race UVPPh7235.

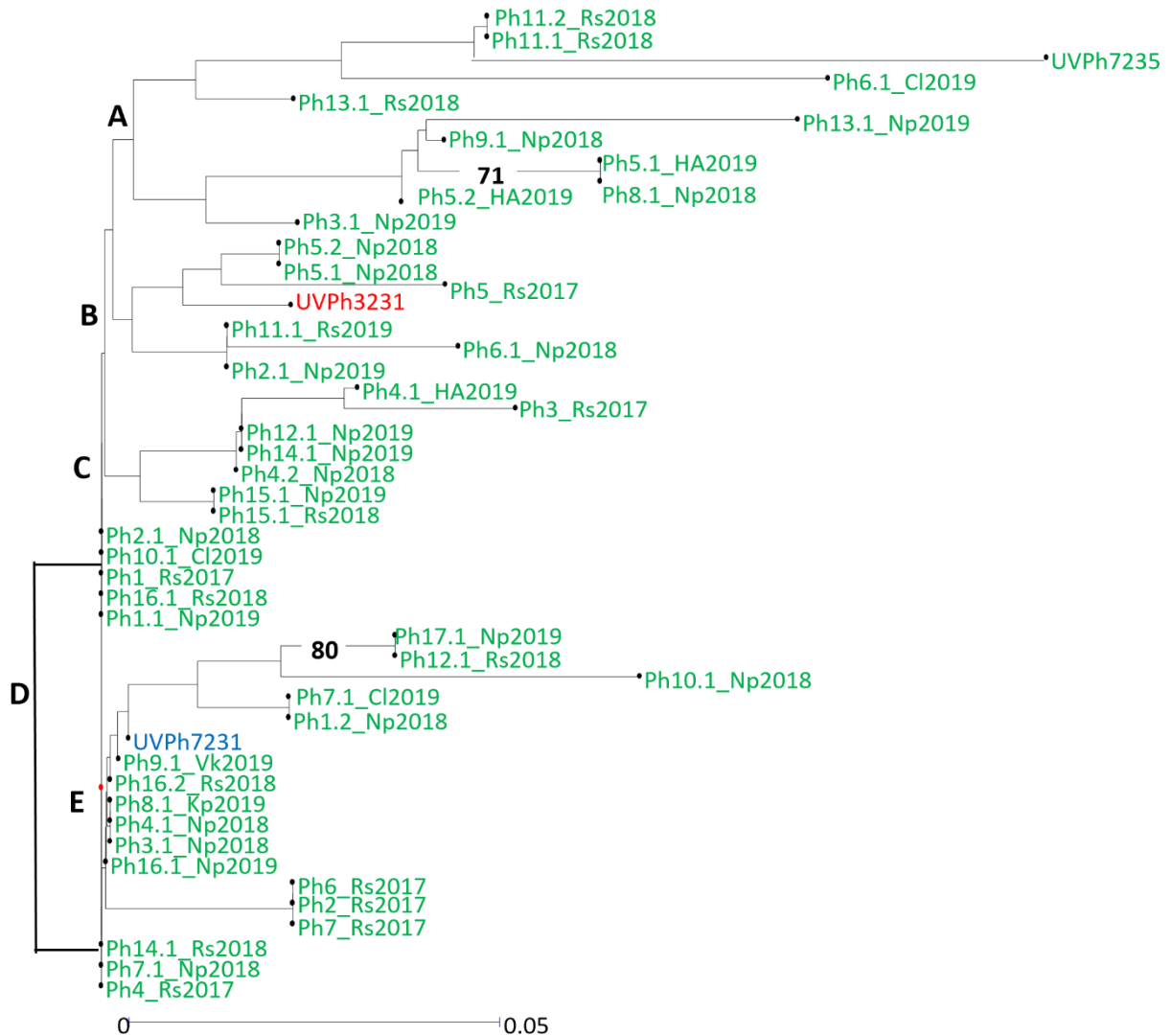
Finally, clade E contained most of the isolates, all showing 95% genetic similarity to each other. In this clade, race UVPPh7231 with virulence to *Rph12* and avirulence to *Rph3*, grouped with 14 field isolates that typed as race UVPPh7235, namely Ph17.1\_Np2019, Ph12.1\_Rs2018, Ph10.1\_Np2018, Ph7.1\_Cl2019, Ph1.2\_Np2018, Ph9.1\_Vk2019, Ph16.2\_Rs2018, Ph8.1\_Kp2019, Ph4.1\_Np2018, Ph3.1\_Np2018, Ph16.1\_Np2019, Ph6\_Rs2017, Ph2\_Rs2017 and Ph7\_Rs2017.

### 4.2.3 Division of the South African *Puccinia hordei* isolates into sub-populations

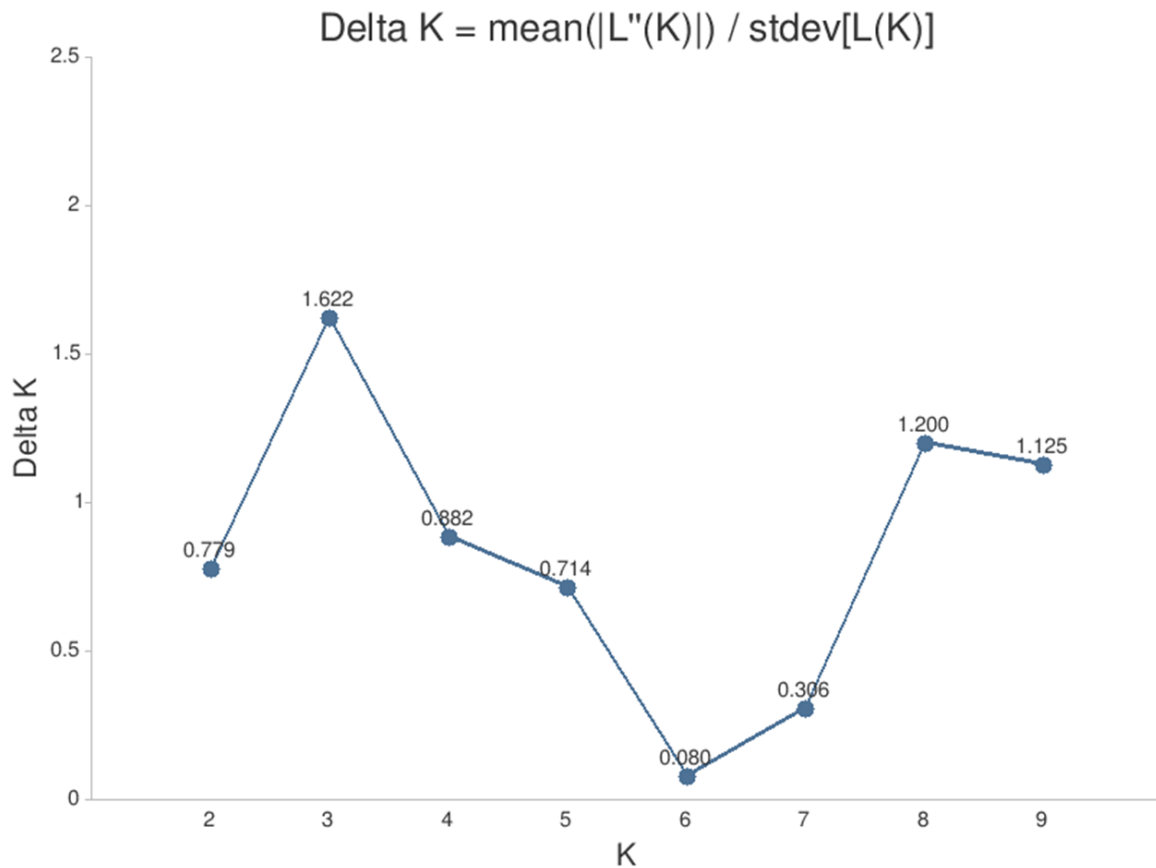
STRUCTURE analysis was used to divide the 33 MLGs into the optimal number of sub-populations. The Bayesian analysis conducted by the STRUCTURE software, used *K*-values that ranged from *K* = 1 to *K* = 10. The optimal *K* value for the 33 MLGs was identified as *K* = 3, depicted by a high  $\Delta K$  value of 1.622 (Figure 4.9), while *K* = 8 was also suggested, however with a lower  $\Delta K$  value of 1.200.

When the 33 MLGs isolates were divided into sub-populations, no clear division of the isolates into the three sub-populations was found (Figure 4.10). Instead, all MLGs were admixed for all three sub-populations. Sub-division of the isolates from *K* = 4 to *K* = 10 did not improve the grouping of the individuals into sub-populations (Figure 4.11), indicating that all individuals most probably formed part of a single population (*K* = 1).

The placement of the 48 *Ph* isolates into the five clades of the Darwin tree was used to perform the AMOVA analysis (Table 4.8). While the genetic differentiation of the isolates within the five clades was significant as indicated by a  $F_{ST}$  value of 0.423 ( $P < 0.001$ ), the AMOVA analysis supported the STRUCTURE results. The AMOVA showed that the main sources of variation were between isolates within the clades (57.607%) and between groups within the clades (45.418%), whereas the least variation (-3.025%) occurred between the clades themselves. This confirmed that the South African *Ph* isolates probably formed a single sub-population with little genetic variation between the isolates.

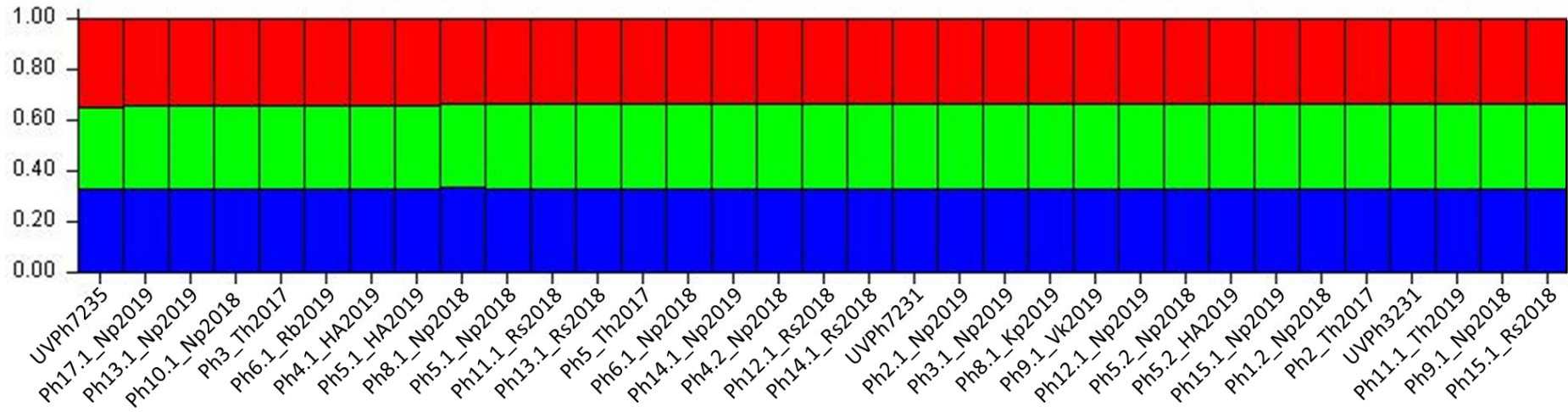


**Figure 4.8** Phylogram of 48 *Puccinia hordei* isolates collected in 2017, 2018 and 2019 from Napier (Np), Hemel and Aarde Vallei (HA), Klipdale (Kp), Riviersonderend (Rs), Voorstekoep (Vk), Caledon (Cl) and Greytown (UVPh7235, 2015), South Africa. Bootstrap values greater than 70 are shown on the branches. The phylogram consisted of five genetic clades labelled A-E. Race UVPh3231 is indicated in red, UVPh7231 in blue and UVPh7235, including the field isolates that typed as UVPh7235, in green.

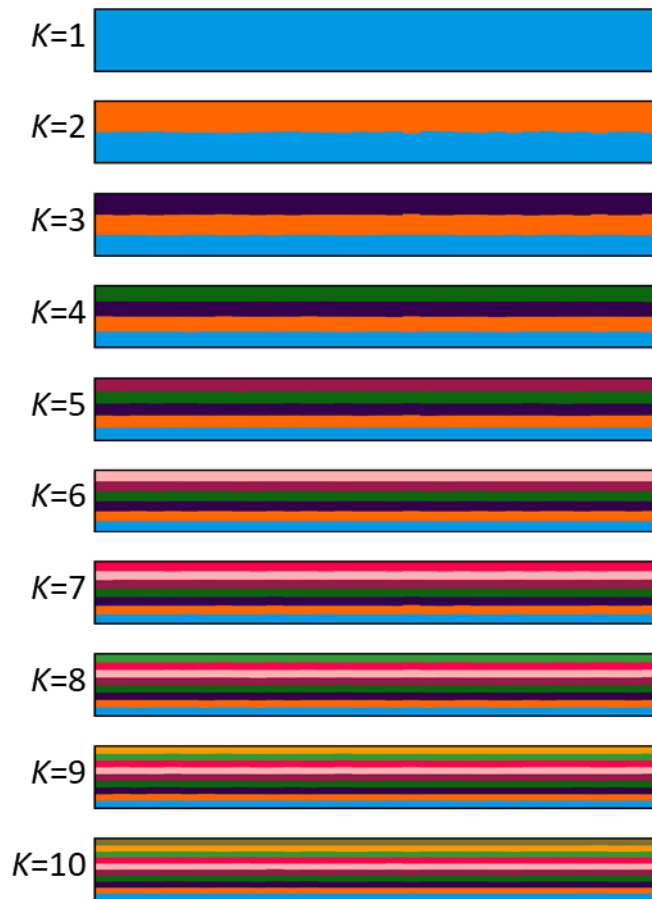


**Figure 4.9** A figure illustrating  $\Delta K$  ( $\text{mean}(|L''(K)|) / \text{stdev}[L(K)]$ ) to determine the optimal  $K$ -value for 33 South African *Puccinia hordei* (*Ph*) multi-locus genotypes (MLGs). The optimal number of sub-populations was determined from  $K$ -values ( $K=1$  to  $K=10$ ) with a 10 000 burn-in period length and 10 000 Monte Carlo Markov Chain (MCMC) repetitions after burn-in with 10 iterations for each  $K$ -value, according to Evanno *et al.* (2005).

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**Figure 4.10** Cluster analysis of 33 South African *Puccinia hordei* (*Ph*) multi-locus genotypes (MLGs) into sub-populations based on  $K=3$  as calculated using STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) software. The clone corrected dataset was used for analysis.



**Figure 4.11** Subdivision of *Puccinia hordei* (*Ph*) isolates representing 33 multi-locus genotypes (MLGs) within the South African population into sub-populations using STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) at different  $K$ -values as clustered and averaged using CLUMPAK (Kopelman *et al.*, 2015). The clone corrected dataset was used for analysis.

**Table 4.8** Analysis of molecular variance (AMOVA) of 48 *Puccinia hordei* (*Ph*) race isolates. The AMOVA was based on the division of the isolates into the five clades of the Darwin phylogram.

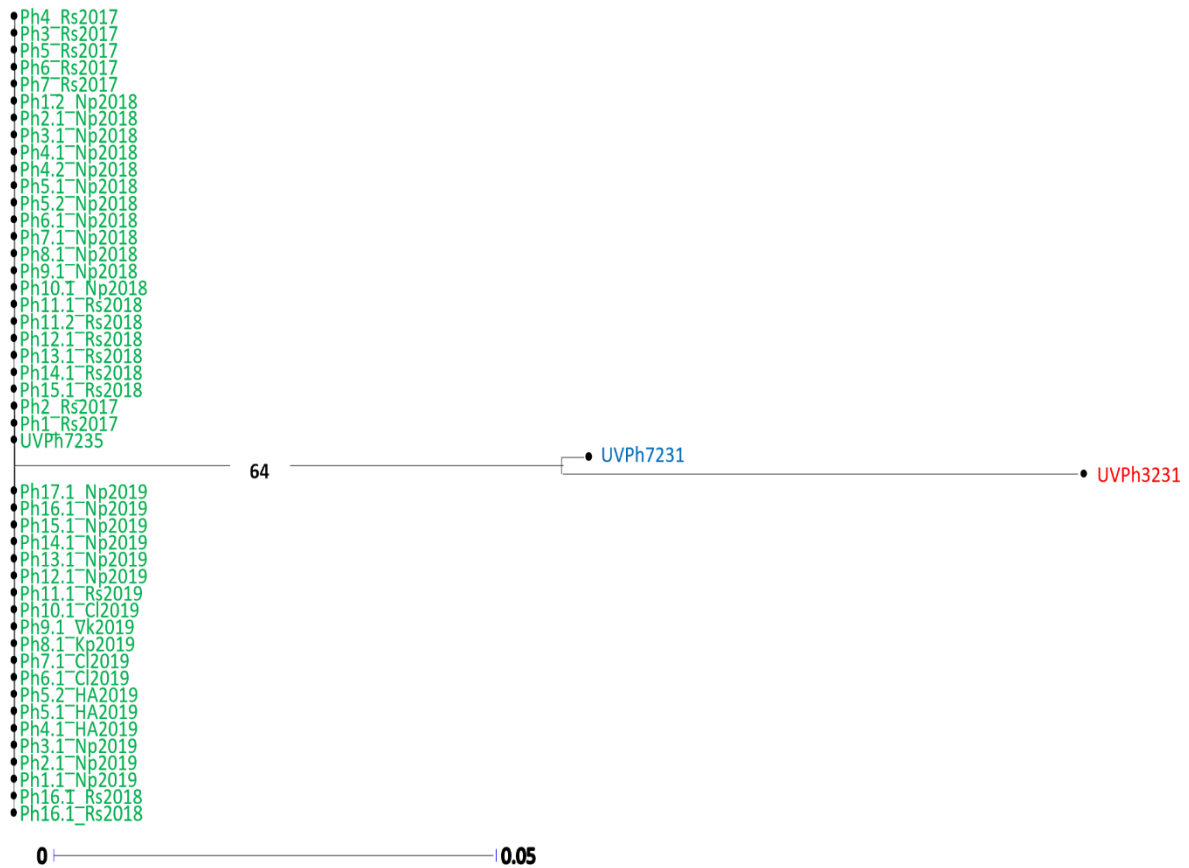
Source of variation	Sum of squares	Variance components	Percentage variation
Among clades	11.671	-0.048	-3.025
Among groups within clades	13.137	0.719	45.418
Within clades	18.650	0.912	57.607
Total	43.458	1.583	

#### 4.2.4 Correlation between the phenotypes and genotypes of the South African *Puccinia hordei* isolates

A dendrogram depicting the phenotypes of all *Ph* races and field isolates was prepared using the binary dataset (Figure 4.12). Race UVPh3231 with avirulence to *Rph3* and *Rph12* was phenotypically distant from all other isolates and races. The remaining isolates shared at least 90% phenotypic similarity. Race UVPh7231, which gained virulence for *Rph12* (avirulent to *Rph3*) was intermediate to race UVPh7235, which additionally gained virulence for *Rph3*. All field isolates with virulence to *Rph3* and *Rph12* clustered with race UVPh7235.

To determine whether there was a correlation between the genotypes (Figure 4.8) and phenotypes (Figure 4.12) of the *Ph* races and isolates, the derived genetic distance matrices were compared. A non-significant weak negative correlation ( $r=-0.034$ ;  $P=0.56$ ) confirmed that no correlation was evident.

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**Figure 4.12** A dendrogram representing the phenotypic variation of 48 *Puccinia hordei* isolates collected in 2017, 2018 and 2019 from Napier (Np), Hemel and Aarde Vallei (HA), Klipdale (Kp), Riviersonderend (Rs), Voorstekop (Vk), Caledon (CI) and Greytown (UVPPh7235, 2015), South Africa. The bootstrap value of 64 is shown on the branch. Race UVPPh3231 is indicated in red, UVPPh7231 in blue and UVPPh7235, as well as all the isolates that typed as UVPPh7235, in green.

## Chapter 5 Discussion

In SA, barley is regarded as the second most important small grain crop after wheat (Anonymous, 2019), emphasizing its high value in the agricultural industry. Barley production has expanded since the late nineties from the WC province to the irrigation areas of the NC and parts of NW and the FS provinces. Production under irrigation was required to meet the increasing demand for barley with a more consistent and high malting quality. This necessitated the development of high yielding barley varieties that are specifically adapted to production under irrigation.

Globally, numerous diseases have been reported on barley, with leaf rust being considered to have the highest epidemic potential (Park *et al.*, 2015). This is due to historic reported yield losses of up to 62% under severe epidemic outbreaks (Cotterill *et al.*, 1992). Under normal conditions, losses vary between 15% and 25% (Whelan *et al.*, 1997). Yield losses within this range were recorded in many different barley production regions of the world, such as Australia (Waterhouse, 1927; Cotterill *et al.*, 1992; 1995; Park *et al.*, 2015), the Czech Republic (Dreiseitl and Steffenson, 2000), Europe (Sandhu *et al.*, 2016), Ethiopia (Semeane *et al.*, 1996), New Zealand (Arnst *et al.*, 1979), SA (Van Niekerk *et al.*, 2001b), the UK (Jenkins *et al.*, 1972; Melville *et al.*, 1976) and the USA (Melville *et al.*, 1976; Griffey *et al.*, 1994). In SA, resistance to *Ph* has been listed as a major breeding priority, together with increased yield potential, scald resistance and brewing quality (Van Niekerk *et al.*, 2001a). Breeding for disease resistance in crops has become more important and is preferred over chemical control to provide an eco-friendly environment, while saving on input costs. In SA, barley leaf rust is chemically controlled through a combination of seed treatment and foliar fungicide application.

There is a lack of knowledge of *Ph* in SA. The study by Van Niekerk *et al.* (2001a) was the last comprehensive race survey of *Ph* in SA reporting on isolates collected between 1994 and 1998. The catalogued *Rph*-resistance genes used then were *Rph1* to *Rph12*. Of these genes, *Rph2*, *Rph3*, *Rph6*, *Rph7* and *Rph9* were effective (low seedling ITs) to the dominating races SAPH 3231 (synonym UVPh3231) and SAPH 7231 (UVPh7231).

## DISCUSSION

To date, a total of 28 *Rph*-resistance genes have been catalogued worldwide (Park *et al.*, 2015; Kavanagh *et al.*, 2017; Ziems *et al.*, 2017; Martin *et al.*, 2020; Mehnaz *et al.*, 2021). The response of these *Rph*-resistance genes to South African *Ph* races has not been determined since the study by Van Niekerk *et al.* (2001a). Globally, the number of catalogued *Rph*-resistance genes available to breeders has decreased rapidly through pathogen adaptation. Plant pathologists and barley breeders need to work continuously to characterise existing and discover novel *Rph* genes, which can be used in resistance breeding.

This study serves as the current South African report on barley leaf rust occurrence, pathogenic variability (phenotypic and genotypic), variety response and effectiveness of catalogued resistance genes in the seedling and adult plant stages. Barley leaf rust has not yet reached epidemic levels in SA. However, the disease is endemic to the dryland production areas of the WC province. In SA, dryland area production outweighs irrigation areas in terms of hectares planted with an estimated 100 000 hectares (<http://www.sagis.org.za>; accessed 05 April 2021) as of 1998, making the occurrence of *Ph* one of the major constraints in barley production. Van Niekerk *et al.* (2001a) stated that the repeated, mostly preventative foliar fungicide applications to control scald caused by *Rhynchosporium commune* (syn *R. secalis*) (Oudem) J. J. Davis, in the WC result indirectly in the chemical control of barley leaf rust. This practice, in combination with the abnormally dry 2018 and 2019 production seasons, could explain the low incidence of barley leaf rust observed in commercial fields during this study.

Barley leaf rust has not yet become a problem in the irrigation production areas of the summer rainfall areas of SA. This is despite the increase in hectares planted with leaf rust susceptible varieties like Overture in the NC, NW, and FS provinces. Most *Ph* isolates characterised in this study therefore originated from unsprayed trial plots at the Roodebloem, Riviersonderend and Napier research stations in the WC province.

Planting of barley in SA is dominated by a few varieties as determined by contractual production agreements between producers and processors of the grain. During the 2019 season, the varieties Hessekwa, Agulhas, Elim and Erica combined accounted for >95% of the hectares planted in the WC (personal communication, Daniel de Klerk, ABInBev).

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Similarly, the ARC-SG has catalogued Agulhas, Hessekwa, and Elim as the first-choice varieties for commercial barley producers in the WC region for the past four production years (2017 to 2020) (Anonymous, 2017; 2018; 2019; 2020). Through contractual growing of barley in SA, producers are limited in variety choice which contributes to creating a more uniform crop.

An international differential set was used for race typing to compare collected *Ph* isolates with those from previous reported papers (Van Niekerk *et al.*, 2001a; Elmansour *et al.*, 2017) as a frame of reference on *Ph* phenotypic diversity. Only one race (UVPPh7235) was described from field isolates collected from mainly the WC production area between 2017 and 2019. This was not unexpected with barley being cultivated as a small grain crop in SA with a likely low genetic diversity considering the number of varieties and area planted as well as an anticipated low number of deployed *Rph*-resistance genes.

The barley leaf rust pathogen is more likely to be reliant on sexual recombination for the completion of its life cycle in Mediterranean areas (with dry summers), which may result in the regular appearance of more virulent races (Reinhold and Sharp, 1982). According to Clifford (1985) as further described by Van Niekerk *et al.* (2001a), cohabitation of the alternate host (*Ornithogalum*) and *Hordeum* spp. may play an important role in the epidemiology of *Ph* in SA. Fifty-four *Ornithogalum* spp. have been reported to occur in SA and also different *Hordeum* spp., especially in the WC province, which could contribute to pathogenic variation and survival of the barley leaf rust pathogen (Van Niekerk *et al.*, 2001a).

In regions like Australia, with similar production conditions to SA, the presence of *O. umbellatum* (Star of Bethlehem) allows for the completion of the pathogen's life cycle (Park *et al.*, 2015). Surprisingly, the telial stage is reportedly more important for the survival of the pathogen in Australia but does not necessarily contribute to the high number of reported races (Park *et al.*, 2015). The telial stage has been implicated to play an important role in both pathogen survival and pathotype variation in Israel (Anikster, 1984).

In the current study, only race UVPPh7235 was detected in SA, while the two historic races UVPPh3231 and UVPPh7231 were not found among the field isolates. This somehow depicted

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conditions common to Europe (Park *et al.*, 2015) where the season and growth of the alternate host and germination of telia do not coincide and are therefore not contributing to pathogen variation.

This points towards the evolution of *Ph* in SA from race UVPPh3231 (first reported in 1994; Van Niekerk *et al.*, 2001a) to race UVPPh7231 (1998; Van Niekerk *et al.*, 2001a) and more recently to the current dominating race UVPPh7235, first described from a field isolate detected near Greytown, KZN in 2015. The absence of race UVPPh3231 from field isolates is not surprising as this race produced low seedling ITs on all the current SA barley varieties. Only Hessekwa and Erica were seedling susceptible to race UVPPh7231.

The large area planted under barley varieties susceptible to race UVPPh7235 may explain the lack of phenotypic variation in the *Ph* pathogen population. Eight of the commercial varieties, including dominant varieties like Hessekwa (58% of hectares planted during 2019, personal communication, Daniel de Klerk, ABInBev), Elim and Erica were MS under field conditions to race UVPPh7235. The only exceptions were Agulhas and Cristalia recommended for production under both irrigation and dryland conditions in the WC province, respectively. These two varieties currently represent the only two barley varieties with low seedling ITs to all three *Ph* races and with confirmed moderate levels of field resistance to UVPPh7235.

A previous report stated that the sexual stage of *Ph* on *Orithinogalum* spp. has not been detected in SA and may further explain the lack of phenotypic variation observed in this study (Van Niekerk *et al.*, 2001a). Results from this study therefore corresponds with Van Niekerk *et al.* (2001a) as the most likely development of *Ph* pathotypes in SA originated from single-step mutations to overcome individual *Rph*-resistance genes.

The IT response data of local barley varieties to the three South African races led to the postulation of two resistance genes. Varieties Hessekwa and Erica are likely to carry *Rph12*, while the varieties Elim, Genie, Kadie, KWS Irina and Oventure are postulated to carry *Rph3*. Although a similar low and high IT pattern was observed for variety Deveron with isolates of the three *Ph* races, the low IT of ;12+ to races UVPPh3231 and UVPPh7231 is not typical of *Rph3* and may therefore represent an unknown resistance gene. The remaining

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two varieties Agulhas and Cristalia both carry unidentified resistance gene(s) with a stronger APR response recorded for Cristalia. This may indicate that these varieties do carry different ASR genes or that Cristalia carries an additional source(s) of APR. However, if of monogenic nature, new *Ph* race(s) are expected to overcome this source(s) of resistance should the two varieties become more dominant in future plantings.

Results further explains the phenotypic diversity of *Ph* in SA with resistance gene *Rph12* being currently the most dominant gene in cultivated barley (varieties Hessekwa and Erica) considering the area planted, followed by *Rph3*. In the paper by Van Niekerk *et al.* (2001a), the susceptible variety Clipper was reported dominant (>95% of area planted) during the timeframe of their study. The first South African variety released with *Rph12*, namely SSG 532, became susceptible to UVPPh7231 shortly after its release in 1997 (Van Niekerk *et al.*, 2001a). Evaluation of barley varieties under field conditions in this study showed consistently moderate to high levels of susceptibility recorded for the varieties Deveron, Elim, Erica, Genie, Hessekwa, Kadie, KWS Irina and Overture in nurseries planted at Greytown and Napier in all tested years illustrating the damaging potential of *Ph* race UVPPh7235.

According to the individual percentage data obtained from Mr Daniel de Klerk (personal communication, ABInBev), the area planted with individual varieties under dryland in the WC during the 2019 season was dominated by Hessekwa (58%) followed by Agulhas (18%), Elim (14%), Erica (8%) and Kadie (2%) and under irrigation Overture (79%), Cristalia (11%), KWS Irina (5%), Genie (3%) and Deveron (2%). This drew a current picture that 82% of barley hectares planted in the WC and 89% under irrigation during 2019 were under *Ph* susceptible varieties considering their seedling and adult responses to *Ph* race UVPPh7235. With no APR resistance observed for most of these varieties, the epidemic potential of barley leaf rust remains high. Outbreaks of barley leaf rust are expected under favourable conditions for *Ph* when fungicides are not applied or when the timing of applications are too late due to unforeseen circumstances like weather conditions or a lack of availability of chemicals. Interestingly were the slightly more resistant adult plant infection responses recorded for varieties Hessekwa, Elim and Deveron to UVPPh7235 in the greenhouse trials. This was inconsistent with the results from field trials over three seasons (2017 to 2020).

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Whether this can be explained by the more moderate temperatures that were applied under greenhouse conditions or the single inoculation cycle versus consecutive cycles of infection possible under field evaluation, needs to be determined. From the results it appeared that in the absence of chemical control, *Ph* will remain a significant threat in the production of high yielding barley of acceptable malting quality with the potential to negatively impact on the economy of the local barley industry.

Considering the seedling ITs recorded on the international set of differential lines, the data obtained in this study is in line with that reported by Van Niekerk *et al.* (2001a), only differing in the addition of virulence to the resistance gene *Rph3*. Differential lines with resistance genes *Rph6*, *7*, *9*, *13*, *14*, *15/16*, *17*, *18*, *21*, *22*, *26* and *Rph27* produced low seedling ITs to isolates of all three *Ph* races. Field data obtained for international lines with *Rph6*, *7*, *9*, *13* and *Rph14* to UVPPh7235 were in accordance with the seedling data, producing MR and R host responses. Despite the addition of pathotype UVPPh7235 from this study, options are available to incorporate some of the effective ASR genes into new varieties to control barley leaf rust in SA. Park *et al.* (2015) reported that countries like New Zealand and Australia detected virulence to *Rph3*. Similarly, Israel, Morocco and the USA reported virulence to *Rph7*, but this gene remains effective in Europe (Park *et al.*, 2015) and SA. Resistance genes *Rph11* and *Rph14* were regularly reported as ineffective in most parts of the world with the exclusion of Australia (Park *et al.*, 2015), while *Rph14* is regarded as effective in SA when considering the observed resistant seedling and field response with variety PI 584760.

However, the presence of more than one resistance gene in a differential line may impact the expression of seedling ITs and complicate the interpretation of the results. For example, several international lines reportedly carry the *Rph2* resistance gene, either singly or in combination with other resistance genes. These include Peruvian (*Rph2*), Bolivia (*Rph2* + *Rph6*), Quinn (*Rph2* + *Rph5* + *Rph27*) and Ricardo (*Rph2* + *Rph21*) which produced low seedling ITs to all local race isolates. However, Reka 1 (*Rph2* + *Rph19*) produced high seedling ITs to all *Ph* race isolates indicating virulence for both *Rph2* and *Rph19*. Peruvian thus seems to carry an additional resistance gene to the *Ph* isolates used in this study. Virulence for *Rph19* in Reka 1 (*Rph2* + *Rph19*) is supported by the high seedling IT response

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recorded for the differential line Prior (*Rph19*) (Hickey *et al.*, 2011) as reported in Australia (Park *et al.*, 2015). Discrepancies found between differential lines can only be resolved through the development of NILs, which motivated the development of the BW introgression lines (Martin, 2018; Martin *et al.*, 2020).

The development of NILs carrying only one *Rph* gene in a universal susceptible background, as with the BW introgression lines, is important and should allow for accurate characterisation of resistance gene responses and assist in avirulence/virulence description of *Ph* isolates. The BW introgression lines were developed by backcrossing donor sources of resistance genes *Rph1* to *Rph15* into the barley variety BW (PI 483237) (Chicaiza *et al.*, 1996; Caffarel, 2005; Martin, 2018; Martin *et al.*, 2020). The international lines, when compared to the BW lines with both carrying resistance genes *Rph1* to *Rph15*, had similar responses. As expected, discrepancies occurred implying that a few *Rph*-resistance genes with low ITs in the international lines, were not effective to SA races. Where discrepancies occurred between the international differential and BW lines, the latter is considered a more accurate reflection of virulence.

Using the BW lines, virulence for *Rph2* was confirmed through the high seedling IT produced on BW743. This was in further support that the international line Peruvian (*Rph2*) is likely to carry an additional resistance gene(s) to SA *Ph* isolates. Further observed discrepancies included BW756 (*Rph6*) and BW760 (*Rph9*) that produced high seedling ITs compared to low seedling ITs on the international lines Bolivia (*Rph2* + *Rph6*), Bolivia/Gus (*Rph6*) and Abyssinian (*Rph9*). The discrepancy found between the two NILs with *Rph6*, Bolivia/Gus and BW756, requires further clarification. The slightly higher seedling IT and susceptible field response for BW686 (*Rph14*, IT=12+, 70S) compared to the data recorded for PI 584760 (*Rph14*, IT=;12C, 20 to 30 MRR), indicates an incomplete ASR response for this resistance gene in the BW background. A further discrepancy observed was between Egypt 4 and BW759, both carrying *Rph8*, and with high seedling ITs. However, the field response observed for Egypt 4 do indicate the presence of APR in the Greytown trials. Similarly, the variety Prior (P) carrying *Rph19* were found more resistant at Napier when compared to Greytown. Further extension of the BW lines to include *Rph16* to *Rph28* and

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other newly catalogued *Rph*-resistance genes may present a valuable resource for use in future studies.

APR sources of rust resistance typically confer susceptibility during the seedling stage, but later show a reduction in the size and number of rust pustules present on the leaves as the plants reach the adult plant growth stages (Parlevliet and Kievit, 1986; Hickey *et al.*, 2011; Rothwell *et al.*, 2019). This type of resistance has been observed towards different rust pathogens and include the well characterised *Lr34/Yr18/Sr57* resistance gene in wheat that contributes additive resistance to different rust pathogens for over 50 years (Dyck and Samborski, 1982; Dyck, 1987; Krattinger *et al.*, 2009).

As expected, the varieties Flagship (*Rph20*) (Hickey *et al.*, 2011) and Yerong (*Rph2* + *Rph23*) (Rothwell *et al.*, 2019) were susceptible at seedling stage. The results for Yerong is in further support of *Rph2* virulence among local *Ph* isolates and in accordance with the results obtained with Reka 1 (*Rph2* + *Rph19*) and line BW743 (*Rph2*). However, Baronesse, reportedly containing both *Rph20* and *Rph24* (Rothwell *et al.*, 2019), produced a low seedling IT to all the *Ph* isolates which did not allow for evaluation of the two APR genes in combination. The seedling IT results further revealed that variety Lenka (*Rph20* + *Rph23* + *Rph24*) is postulated to carry the ASR gene *Rph3*. This postulation support results in a study by Dracatos *et al.* (2021). Studying the combined effect of the three APR genes in Lenka thus requires access to *Rph3* virulent races like UVPPh7235. Despite the combination of all three APR genes, Lenka was susceptible under field conditions and moderately susceptible under greenhouse conditions to race UVPPh7235. As expected, Lenka produced a strong resistance response in greenhouse evaluation to isolates of *Ph* races UVPPh3231 and UVPPh7231, attributed to the presence of *Rph3*. The susceptible field response of Lenka to *Ph* race UVPPh7235 contradicts the conclusion reported in Dracatos *et al.* (2021) that even though *Rph3* was ineffective to a *Ph* pathotype used in their Cobbitty field nursery, an almost immune field response was obtained due to the combined effect of the three APR genes.

The results from this study further contradicts the report by Ziems *et al.* (2017) that the combination of all three APR genes provided a higher level of resistance compared to *Rph20* and *Rph24* due to the epistatic effect of *Rph20* on the combination of *Rph23* and

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*Rph24* (Rothwell *et al.*, 2019). The APR genes *Rph20* in Flagship and *Rph23* in Yerong produced MS to S and MS responses to UVP7235 under field conditions, respectively. However, a stronger resistance response (MRR) was produced by variety Yerong (*Rph2* + *Rph23*) under greenhouse evaluation compared to field evaluation (MS). *Rph20* has been reported to be expressed at temperatures ( $18\pm 2^{\circ}\text{C}$ ) similar to the experimental greenhouse conditions applied in this study. Flagship produced an MS APR response to all three *Ph* race isolates under greenhouse conditions.

The understanding of a pathogen population's genetic structure provides detail on its ability and potential to overcome the genetic resistance of the host (McDonald and Linde, 2002). *Puccinia hordei* genotypic studies in Australia by Karaoglu and Park (2014) and Sandhu *et al.* (2016) indicated significant genetic diversity among *Ph* isolates. In Australia, factors contributing to the genetic diversity of *Ph*, include long distance spore migration and mutations. Furthermore, *Ph* is the only cereal rust pathogen in Australia reported to undergo sexual recombination in the presence of the alternate host, *O. umbellatum* (Park *et al.*, 2015). This is expected to contribute to a more diverse *Ph* population with an anticipated increase in pathotypes with diverse virulence combinations.

While microsatellite marker studies were previously successfully used to distinguish amongst race isolates of different *Puccinia* species in SA (Visser *et al.*, 2009; 2012; Terefe *et al.*, 2014; Boshoff *et al.*, 2018; 2019), the genetic diversity of *Ph* isolates has not been studied before. Results from this study therefore provide the first estimate of *Ph* genetic diversity. The *Ph* isolates that were included, represented collections made during the 2017 to 2019 barley growing seasons and included two reference isolates dating back to collections made in the 1990s as well as one isolate, Pt3\_Gt2015 (UVP7235), collected from a barley trial planted near Greytown in 2015. Although the limited number of *Ph* isolates did not allow for a comprehensive population study, the results do provide a glimpse into the development of *Ph* in SA.

Selected microsatellite markers, reported as polymorphic by Karaoglu and Park (2014), portrayed low levels of polymorphism among local *Ph* isolates, indicating a narrow genetic base. All 48 isolates were successfully grouped into five different clades in the DARwin tree. While the placement of the individuals in relation to each other were significant ( $F_{ST} =$

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0.423;  $P < 0.001$ ), all isolates were less than 10% dissimilar. Several of the *Ph* isolates were identical to each other for all 20 microsatellite markers that were used. De Meeus and Balloux (2005) suggested that in a clonal lineage like the South African *Ph* population,  $F_{ST}$  values will not pass 0.5.

STRUCTURE analysis failed to divide the isolates into well-defined sub-populations, but rather indicated that all isolates were admixed for all three sub-populations. This indicated that the optimal number of South African *Ph* sub-populations is  $K = 1$ . AMOVA analysis supported this conclusion when the highest percentage (57%) of genetic variation was attributed to the genetic variation between *Ph* isolates within each sub-clade, while 0% could be attributed to genetic variation between the five sub-clades of the phylogram. This STRUCTURE result was supported by Halkett *et al.* (2005) who said that STRUCTURE analysis is unable to successfully characterise a highly clonal population by dividing the isolates into sub-populations.

Hardy-Weinberg Equilibrium (HWE) is valuable in population genetics and states that allele frequencies will be constant in a population, unless disturbed by evolutionary forces such as genetic drift, natural selection, mutation, gene flow, a population bottleneck and non-random mating (Edwards, 2008; Graffelman *et al.*, 2017). In the current study, the mean observed heterozygosity ( $H_O$ ) was higher than the expected ( $H_E$ ) which in turn influenced the mean inbreeding coefficient ( $F_{is}$ ) value resulting in a significant negative value. A negative  $F_{is}$  value indicates an excess of heterozygotes which is common in clonal lineages (Balloux *et al.*, 2003; Bengtsson, 2003). The average gene diversity was low (0.288), as was the allelic richness (0.287). Together with significant positive values for linkage disequilibrium, it all exhibited a deviation from the expected HWE, meaning that non-random mating has occurred, leading to a clonal *Ph* population. This is common to *Puccinia* species such as *Pt* (Kolmer *et al.*, 2019), *P. striiformis* f.sp. *tritici* (*Pst*) (Cheng and Chen, 2014) and *Pgt* (Visser *et al.*, 2019).

Finally, no significant correlation between the genotypes and phenotypes of the *Ph* isolates was found. This was evident in the phylogram where race UVPPh7235 field isolates were genetically very similar to the UVPPh3231 and UVPPh7231 control isolates. All the results were in accordance with the initial hypothesis of a clonal *Ph* population consisting of a

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single genetic lineage with low genetic variation within, which most probably developed from a single original founding isolate in SA. It appears that single-step mutation is the only contributing factor to the pathogenic variation of *Ph* due to selection pressure created by cultivated barley varieties. This corresponds with the phenotypic data where variation in the virulence profiles among *Ph* isolates was only detected for *Rph3* and *Rph12*, thus painting a vivid picture that the mode of reproduction of *Ph* in SA is likely to be only asexual.

Similar results were reported in a study from Australia, as *Ph* race 5457P<sup>+</sup> was believed to have originated from race 5453P<sup>+</sup> due to step-wise mutations as virulence was detected for *Rph19* followed by *Rph3* (Sandhu *et al.*, 2016). Despite the presence of the sexual cycle in Australia, 0% genetic dissimilarity was observed between *Ph* race isolates 201P<sup>+</sup> and 201P<sup>-</sup> using molecular marker repeat motif (GACA)<sub>4</sub>. Race 201P<sup>+</sup> reportedly arose through a step-wise mutation with added virulence for *Rph19* in 201P<sup>-</sup> (Sandhu *et al.*, 2016).

Therefore, this is all in support of a single founder isolate which underwent single-step mutation and in further, explains the initial presence of race UVPPh3231 in SA. Contributing factors could be the restriction in the commercial varieties planted by local producers and an anticipated relatively low genetic variation among these varieties. The latter is to a certain extent determined by the relative uniform and small production area allowing for a more uniform and small gene pool. This was also detected in the phenotypic study of *Ph* isolates with all the field isolates collected since 2017 in SA typed as race UVPPh7235 revealing restricted variation in both the phenotype and genotype among local *Ph* isolates. This is in further support of the likely absence of the sexual stage of *Ph* in SA. The phenotypic and genotypic uniformity observed among *Ph* isolates in this study provide no evidence of recent foreign incursions of *Ph* in SA. This has been proposed following results from phenotypic and genotypic studies for other *Puccinia* species (Terefe *et al.*, 2014; Visser *et al.*, 2016). Future studies should include *Ph* isolates from different continents as control isolates to provide a better estimate of genetic variation when comparing with South African *Ph* race isolates.

## Chapter 6 Conclusions and Recommendations

### 6.1 Conclusions

This study provides the virulence profiles of *Ph* races reported on barley in SA, which includes the newly identified race UVPh7235 typed from all recently collected field isolates in combination with isolates representative of the two historic races UVPh3231 and UVPh7231. From the presented data, it appears that the use of seed treatments like Redigo® in combination with foliar fungicide application in SA (Daniel de Klerk - personal communication, January 2021) is justified when the MS to S leaf rust responses of most of the available barley varieties are considered. The barley variety response data revealed that leaf rust outbreaks is likely to occur under environmental conditions favourable for disease development in the absence of timely chemical control. The discrepancies observed in the seedling ITs between some of the differential lines, supposedly carrying the same *Rph*-resistance genes, indicate the presence of uncharacterised sources of resistance to South African *Ph* isolates. The study revealed low levels of phenotypic and genotypic variation among *Ph* isolates collected over three barley production seasons. A high genetic similarity between isolates of the three *Ph* races and recently collected field isolates supports the evolution of the pathogen in SA through step-wise mutation. This confirms that the reproduction of *Ph* is likely to be asexual as confirmed by molecular marker analysis which is in accordance with observations made during surveys of potential alternate host plants by Van Niekerk *et al.* (2001a).

Phenotypic and genotypic data from this study is considered representative of *Ph* in SA. Isolates of each *Ph* race were used to determine the response of barley varieties in both seedling and adult plant stages under greenhouse and field conditions. The results set the foundation for barley breeders in their efforts to develop and release new varieties resistant to *Ph*. The variety response data should assist barley producers in managing the disease through variety choice and optimising timing of chemical applications on varieties with MS and S responses, which should contribute to improve yield and grain quality.

## 6.2. Recommendations

Regular pathogenicity surveys assessing *Ph* virulence are required as newly designated *Rph*-resistance genes become available. This will support breeding for resistance to *Ph* and assist in the release of barley varieties with effective levels of field resistance. Data on barley variety responses should be regularly updated to include data for new releases, as well as the impact that new races of the pathogen may have on the response of already commercialised varieties. The search for novel sources of *Rph*-resistance and the development of closely linked molecular markers should be encouraged through industry funding. Studies on the susceptibility of alternate host species to *Ph* in SA, the effect of incubation temperature on resistance gene expression under controlled conditions and monitoring of *Ph* isolates for potential resistance to regularly applied fungicides, are some outstanding aspects of further research interest. Lessons learnt from other cereal rusts in SA include the risk of incursions and the severe impact that such isolates may have on variety response. With the recent expansion of barley production into African countries in the Southern African region, bridging the gap in production between SA and North-Africa, *Ph* incursion events are considered more likely. Surveillance studies remain therefore key in early detection of new more virulent pathogen isolates and early warning, including their impact on variety response to producers.

## References

- Agapow PM, Burt A. 2001. Indices of multilocus linkage disequilibrium. *Molecular Ecology Notes* 1:101-102.
- Anikster Y. 1982. Alternate hosts of *Puccinia hordei*. *Phytopathology* 72:733-735.
- Anikster Y. 1984. Parasitic specialization of *Puccinia hordei* in Israel. *Phytopathology* 74:1061-1064.
- Anikster Y, Eilam T, Mittelman L, Szabo LJ, Bushnell WR. 1999. Pycnial nectar of rust fungi induces cap formation on pycniospores of opposite mating type. *Mycologia* 91:858-870.
- Anikster Y, Wahl I. 1979. Coevolution of the rust on *Gramineae* and *Liliaceae* and their hosts. *Annual Review of Phytopathology* 17:367-403.
- Anonymous. 2017. Guideline- production of small grains in the winter rainfall area. ARC-Small Grain. Bethlehem, South Africa.
- Anonymous. 2018. Guideline- production of small grains in the winter rainfall area. ARC-Small Grain. Bethlehem, South Africa.
- Anonymous. 2019. Guideline- production of small grains in the winter rainfall area. ARC-Small Grain. Bethlehem, South Africa.
- Anonymous. 2020. Guideline- production of small grains in the winter rainfall area. ARC-Small Grain. Bethlehem, South Africa.
- Aradhya MK, Chan HM, Parfitt DE. 2001. Genetic variability in the pistachio late blight fungus, *Alternaria alternata*. *Mycological Research* 105:300-306.
- Arnaud-Haond S, Belkhir K. 2007. GenClone: A computer program to analyze genetic data, test for clonality and describe spatial clonal organization. *Molecular Ecology Notes* 7:15-17.
- Arnst BJ, Martens JW, Wright GM, Burnett PA, Sanderson FR. 1979. Incidence, importance and virulence of *Puccinia hordei* on barley in New Zealand. *Annals of Applied Biology* 92:185-190.

## REFERENCES

- AVCASA. 2021. Croplife South Africa. Available at [www.avcasa.com](http://www.avcasa.com). [05 March 2021].
- Balloux F, Lehmann L, De Meeus T. 2003. The population genetics of clonal and partially clonal diploids. *Genetics* 164:1635-1644.
- Bengtsson BO. 2003. Genetic variation in organisms with sexual and asexual reproduction. *Journal of Evolution Biology* 16:189-199.
- Blattner FR. 2009. Multiple intercontinental dispersals shaped the distribution area of *Hordeum* (Poaceae). *New Phytologist* 169:603-614.
- Borovkova IG, Jin Y, Steffenson BJ. 1998. Chromosomal location and genetic relationship of leaf rust resistance genes *Rph9* and *Rph12* in barley. *Phytopathology* 88:76-80.
- Borovkova IG, Jin Y, Steffenson BJ, Kilian A, Blake TK, Kleinhofs A. 1997. Identification and mapping of leaf rust resistance gene in barley line Q21861. *Genome* 40:236-241.
- Boshoff WHP, Labuschagne R, Terefe T, Pretorius ZA, Visser B. 2018. New *Puccinia triticina* races on wheat in South Africa. *Australasian Plant Pathology* 47:325-334.
- Boshoff WHP, Van Niekerk BD, Pretorius ZA. 2000. Races of *Puccinia graminis* f. sp. *tritici* detected in South Africa during 1991-1997. *South African Journal of Plant and Soil* 17:60-62.
- Boshoff WHP, Visser B, Terefe T, Pretorius ZA. 2019. Diversity in *Puccinia graminis* f. sp. *avenae* and its impact on oat cultivar response in South Africa. *European Journal Plant of Pathology* 155:1165-1177.
- Botstein D, White RL, Skolnick M, Davis RW. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics* 32:314-331.
- Brookes AJ. 1999. The essence of SNPs. *Gene* 234: 177-186.
- Brunner S, Keller B, Feuillet C. 2000. Molecular mapping of the *Rph7* leaf rust resistance gene in barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics* 101:783-788.
- Caffarel JC. 2005. Leaf rust studies in barley using Bowman backcross-derived lines. MSc thesis, Fargo: North Dakota State University, United States of America.

## REFERENCES

- Carmona M, Sautua F, Pérez-Hernández O, Reis EM. 2020. Role of fungicide applications on the integrated management of wheat stripe rust. *Frontiers in Plant Science* 11:733.
- Castro AJ, Gamba F, German S, Gonzalez S, Hayes PM, Pereyra S, Perez C. 2012. Quantitative trait locus analysis of spot blotch and leaf rust resistance in the BCD47 × Baronesse barley mapping population. *Plant Breeding* 131:258-266.
- Chen C, Jost M, Clark B, Martin M, Matny O, Steffenson BJ, Franckowiak JD, Mascher M, Singh D, Perovic D, Richardson T, Periyannan S, Lagudah ES, Park RF, Dracatos PM. 2020. Ancient BED-domain-containing immune receptor from wild barley confers widely effective resistance to leaf rust. Cold Spring Harbor Laboratory, bioRxiv.
- Cheng P, Chen X. 2014. Virulence and molecular analyses support asexual reproduction of *Puccinia striiformis* f. sp. *tritici* in the US Pacific Northwest. *Phytopathology* 104:1208-1220.
- Chicaiza O, Franckowiak JD, Steffenson B. 1996. New sources of resistance to leaf rust in barley. Proc. International Oat Conference, 5th, International Barley Genetics Symposium, 7th, Saskatoon, July 30–August 6. Saskatoon, Sask.: Mister Print Production Limited.
- Chu CG, Friesen TL, Xu SS, Faris JD, Kolmer JA. 2009. Identification of novel QTLs for seedling and adult plant leaf rust resistance in a wheat doubled haploid population. *Theoretical and Applied Genetics* 119:263-269.
- Clifford BC. 1972. The histology of race non-specific resistance to *Puccinia hordei* Otth. in barley. In: Proceedings: 3<sup>rd</sup> European and Mediterranean Cereal Rusts Conference 1:75-79.
- Clifford BC. 1985. The formae speciales. In: *The Cereal Rusts, Vol. II: Diseases, Distribution, Epidemiology, and Control*. Roelfs AP, Bushnell WR (eds). Florida: Academic Press.
- Cotterill PJ, Park RF, Rees RG. 1995. Pathogenic specialization of *Puccinia hordei* Otth in Australia, 1966–1990. *Australian Journal of Experimental Agriculture* 46:127-134.
- Cotterill PJ, Rees RG, Platz GJ, Dill-Macky R. 1992. Effects of leaf rust on selected Australian

## REFERENCES

- barleys. Australian Journal of Experimental Agriculture 32:747-751.
- Critopoulos P. 1956. Perpetuation of brown rust of barley in Attica. Mycologia 48:596-600.
- DAFF. 2019. Department of Agriculture, Forestry and Fisheries, South Africa. Abstract of Agricultural Statistics. Available at <http://www.daff.gov.za> [Accessed 23 May 2021].
- Das MK, Griffey CA, Baldwin RE, Waldenmaier CM, Vaughn ME, Price M, Brooks WS. 2007. Host resistance and fungicide control of leaf rust (*Puccinia hordei*) in barley (*Hordeum vulgare*) and effects on grain yield and yield components. Crop Protection 26:1422-1430.
- Dean R, Van Kan JAL, Pretorius ZA, Hammond-Kosack KE, Pietro AD, Spanu PD, Rudd JJ, Dickman D, Kahmann R, Ellis J, Foster GD. 2012. The top 10 fungal pathogens in molecular plant pathology. Molecular Plant Pathology 13:414-430.
- De Meeus T, Balloux F. 2005. Clonal reproduction and linkage disequilibrium in diploids: a simulation study. Infection Genetics and Evolution 4:345-351.
- Dracatos PM, Khatkar MS, Singh D, Park RF. 2014. Genetic mapping of a new race specific resistance allele effective to *Puccinia hordei* at the *Rph9/Rph12* locus on chromosome 5HL in barley. BMC Plant Biology 14:1598.
- Dracatos PM, Park RF, Singh D. 2021. Validating molecular markers for barley leaf resistance genes *Rph20* and *Rph24*. Plant Disease 105:743-747.
- Dracatos PM, Singh D, Bansal U, Park RF. 2015. Identification of new sources of adult plant resistance to *Puccinia hordei* in international barley (*Hordeum vulgare* L.) germplasm. European Journal of Plant Pathology 141:463-476.
- Drake JW, Charlesworth B, Charlesworth D, Crow JF. 1998. Rates of spontaneous mutation. Genetics 148:1667-1686.
- Dreiseitl A, Steffenson BJ. 2000. Postulation of leaf-rust resistance genes in Czech and Slovak barley cultivars and breeding lines. Plant Breeding 119:211-214.
- Dyck PL. 1987. The association of a gene for leaf rust resistance with chromosome 7D suppressor of stem rust resistance in common wheat. Genome 29:467-469.

## REFERENCES

- Dyck PL, Samborski DJ. 1982. The inheritance of resistance to *Puccinia recondita* in a group of common wheat cultivars. *Canadian Journal of Genetics and Cytology* 24:273-283.
- Edwards AWF. 2008. G.H. Hardy (1908) and Hardy–Weinberg equilibrium. *Genetics* 179:1143-1150.
- Elmansour H, Singh D, Dracatos PM, Park RF. 2017. Identification and characterization of seedling and adult plant resistance to *Puccinia hordei* in selected African barley germplasm. *Euphytica* 213:119.
- Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology* 14:2611-2620.
- Excoffier L, Lischer HEL. 2010. Arlequin suite version 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10:564-567.
- FAOSTAT. 2019. (Statistical Pocketbook) Food and Agriculture Organisation of the United Nations, FAO, Rome. Available at <http://www.fao.org/faostat/en/#home> [Accessed 20 May 2021].
- FAOSTAT. 2020. (Statistical Pocketbook) Food and Agriculture Organisation of the United Nations, FAO, Rome. Available at <http://www.fao.org/faostat/en/#home> [Accessed 20 June 2021].
- Fazlikhani L, Keilwagen J, Kopahnke D, Deising H, Ordon F, Perovic D. 2019. High resolution mapping of *RphMBR1012* conferring resistance to *Puccinia hordei* in barley (*Hordeum vulgare* L.). *Frontiers in Plant Science* 10:640.
- Feuerstein U, Brown AHD, Burdon JJ. 1990. Linkage of rust resistance genes from wild barley (*Hordeum spontaneum*) with isozyme markers. *Plant Breeding* 104:318-324.
- Flor HH. 1956. The complementary genic systems in flax and flax rust. *Advances of Genetics* 8:29-54.
- Flor HH. 1971. Current status of the gene-for-gene concept. *Annual Review of Phytopathology* 9:275-296.
- Franckowiak JD, Jin Y, Steffenson BJ. 1997. Recommended allele symbols for leaf rust

## REFERENCES

- resistance genes in barley. *Barley Genetics Newsletter* 27:36-44.
- Gilmour J. 1973. Octal notation for designating physiologic races of plant pathogens. *Nature* 242:620.
- Gnocato FS, Dracatos P, Karaoglu H, Zhang P, Berlin A, Park RF. 2018. Development, characterisation and application of genomic SSR markers for the oat stem rust pathogen *Puccinia graminis* f. sp. *avenae*. *Plant Pathology* 67:457-466.
- Golegaonkar PG, Park RF, Singh D. 2010. Genetic analysis of adult plant resistance to *Puccinia hordei* in barley. *Plant Breeding* 129:162-166.
- Golegaonkar PG, Singh D, Park RF. 2009. Evaluation of seedling and adult plant resistance to *Puccinia hordei* in barley. *Euphytica* 166:183-197.
- Gonzalez AM, Marcel TC, Nicks RE. 2012. Evidence for a minor gene-for-minor gene interaction explaining non-hypersensitive polygenic partial disease resistance. *Phytopathology* 102:1086-1093.
- Gonzales M, Zamora DM, Huerta ZR, Solano HS. 2013. Efficiency of three fungicides to control leaf rust in malting barley. *Revista Mexicana de Ciencias Agrícolas* 4:1237-1250.
- Goudet J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9.3.
- Graffelman J, Jain D, Weir B. 2017. A genome-wide study of Hardy–Weinberg equilibrium with next generation sequence data. *Human Genetics* 136:727-741.
- GrainSA. 2021. Available at <https://www.grainsa.co.za/pages/industry-reports/production-reports>. [Accessed 31 May 2021].
- Grando S, Macpherson HG. 2005. Food Barley: importances, uses and local knowledge. *Proceedings of the International Workshop on Food Barley Improvement*. 14-17 January 2002, Hammamet, Tunisia. (ICARDA, Aleppo, Syria).
- Graner A, Streng S, Drescher A, Jin Y, Borovkova I, Steffenson BJ. 2000. Molecular mapping of the leaf rust resistance gene *Rph7* in barley. *Plant Breeding* 119:389-392.

## REFERENCES

- Griffey CA, Das MK, Baldwin RE, Waldenmaier CM. 1994. Yield losses in winter barley resulting from a new race of *Puccinia hordei* in North America. *Plant Disease* 78:256-260.
- Groth J, Roelfs A. 1982. Effect of sexual and asexual reproduction on race abundance in cereal rust fungus populations. *Phytopathology* 72:1503-1507.
- Halkett F, Simon JC, Balloux F. 2005. Tackling the population genetics of clonal and partially clonal organisms. *Trends in Ecology and Evolution* 20:194-201.
- Hartl DL, Clark AG. 1997. *Principles of Population Genetics*. Third edition. Sunderland. Sinauer Associate.
- Hickey LT, Lawson W, Platz GJ, Dieters M, Arief VN, German S, Fletcher S, Park RF, Singh D, Pereyra S, Franckowiak J. 2011. Mapping *Rph20*: a gene conferring adult plant resistance to *Puccinia hordei* in barley. *Theoretical and Applied Genetics* 123:55-68.
- Hiratsuka Y, Sato S. 1982. Morphology and taxonomy of rust fungi. In: *The rust fungi*. Scott KJ, Chakravorty AK (eds). New York. Academic Press. 1-36.
- Hulbert S, Pumphrey M. 2014. A time for more booms and fewer busts? Unravelling cereal-rust interactions. *Molecular Plant Microbe Interactions* 27:207-214.
- Ivandić V, Walther U, Graner A. 1998. Molecular mapping of a new gene in wild barley conferring complete resistance to leaf rust (*Puccinia hordei* Otth). *Theoretical and Applied Genetics* 97:1235-1239.
- Jaccoud D, Peng K, Feinstein D, Kilian A. 2001. Diversity arrays: a solid-state technology for sequence information independent genotyping. *Nucleic Acids Research* 29:25.
- Jakob SS, Rödder D, Engeler JO, Shaaf S, Özkan H, Blattner FR, Kilian B. 2014. Evolutionary history of wild barley (*Hordeum vulgare* subsp. *spontaneum*) analysed using multilocus sequence data and paleodistribution modelling. *Genome Biology and Evolution* 6:685-702.
- Jenkins JE, Melville SC, Jemmett JL. 1972. The effect of fungicides on leaf diseases and on yield in spring barley in south-west England. *Plant Pathology* 21:49-58.
- Jin Y. 2011. Role of alternate host *Berberis* spp. in generating new races of *Puccinia graminis*

## REFERENCES

- and *Puccinia striiformis*. *Euphytica* 179:105-108.
- Jin Y, Cui GH, Steffenson BJ, Franckowiak JD. 1996. New leaf rust resistance genes in barley and their allelic and linkage relationships with other *Rph* genes. *Phytopathology* 86:887-890.
- Jin Y, Statler GD, Franckowiak JD, Steffenson BJ. 1993. Linkage between leaf rust resistance genes and morphological markers in barley. *Phytopathology* 83:230-233.
- Johnson R. 1981. Durable resistance - Definition of genetic control and attainment in plant breeding. *Phytopathology* 71:567-568.
- Johnson R. 1984. A critical analysis of durable resistance. *Annual Review of Phytopathology* 22:309-330.
- Johnston PA, Nicks RE, Meiyalaghan V, Blanchet E, Pickering R. 2013. *Rph22*: Mapping of a novel leaf rust resistance gene introgressed from the non-host *Hordeum bulbosum* L. into cultivated barley (*Hordeum vulgare* L.). *Theoretical and Applied Genetics* 126:1613-1625.
- Jost M, Singh D, Lagudah E, Park RF, Dracatos PM. 2020. Fine mapping of leaf rust resistance gene *Rph13* from wild barley. *Theoretical and Applied Genetics* 133:1887-1895.
- Karaoglu H, Park RF. 2014 Isolation and characterization of microsatellite markers for the causal agent of barley leaf rust, *Puccinia hordei*. *Australasian Plant Pathology* 47:47-52.
- Kavanagh PJ, Singh D, Bansal UK, Park RF. 2017. Inheritance and characterization of the new and rare gene *Rph25* conferring seedling resistance in *Hordeum vulgare* against *Puccinia hordei*. *Plant Breeding* 136:908-912.
- Knott DR. 1989. *The Wheat Rusts: breeding for resistance*. Springer-Verlag, Heidelberg, Germany.
- Kolmer JA, Ordonez ME, German S, Morgounov A, Pretorius Z, Visser B, Goyeau H, Anikster Y, Acevedo M. 2019. Multilocus genotypes of the wheat leaf rust fungus *Puccinia triticina* in worldwide regions indicate past and current long distance migration. *Phytopathology* 109:1453-1463.

## REFERENCES

- Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I. 2015. CLUMPAK: A program for identifying clustering modes and packaging population inferences across K. *Molecular Ecology Resource* 15:1179-1191.
- Krattinger SG, Lagudah ES, Spielmeier W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B. 2009. A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science* 323:1360-1363.
- Lewis CM, Persoons A, Bebber DP, Kigathi RN, Maintz J, Findlay K, Sancho VB, Moreno PC, Harrington SA, Kangara N, Berlin A, Garcia R, German SE, Hanzalova A, Hodson DP, Hovmoller MS, Espino JH, Imtiaz M, Mirza JI, Justesen AF, Niks RE, Omrani A, Patpour M, Pretorius ZA, Roohparvar R, Sela H, Singh RP, Steffenson B, Visser B, Fenwick PM, Thomas J, Wulff BBH, Saunders DGO. 2018. Potential for re-emergence of wheat stem rust in the United Kingdom. *Communications Biology* 1:13.
- Lim LG, Gaunt RE. 1986. The effect of powdery mildew (*Erysiphe graminis* f. sp. *hordei*) and leaf rust (*Puccinia hordei*) on spring barley in New Zealand. Epidemic development, green leaf area and yield. *Plant Pathology* 35:44-53.
- Liu J, Kolmer J. 1998. Molecular and virulence diversity and linkage disequilibria in asexual and sexual populations of the wheat leaf rust fungus, *Puccinia recondita*. *Genome* 41:832-840.
- Mammadov JA, Zwonitzer JC, Biyashev RM, Griffey CA, Jin Y, Steffenson BJ, Maroof MAS. 2003. Molecular mapping of leaf rust resistance gene *Rph5* in barley. *Crop Science* 43:388-393.
- Manisterski J. 1989. Physiologic specialisation of *Puccinia hordei* in Israel from 1983 to 1985. *Plant Disease* 73:123-135.
- Manoharachary C, Kunwar IK. 2014. Host–pathogen interaction, plant diseases, disease management strategies, and future challenges: In *Fungal Biology: Future Challenges in Crop Protection Against Fungal Pathogens*. Manoharachary C, Goyal A. (eds). New York. Springer.
- Mantel NA. 1967. The detection of disease clustering and a generalized regression

## REFERENCES

- approach. *Cancer Research* 27:209-220.
- Marcel TC, Aghnoum R, Durand J, Varshney RK, Niks RE. 2007. Dissection of the barley 2L1.0 region carrying the '*Laevigatum*' quantitative resistance gene to leaf rust using near isogenic lines (NILs) and sub-NILs. *Molecular Plant Microbe Interaction* 20:1604-1615.
- Martin M. 2018. Development of barley introgression lines carrying the leaf rust resistance genes *Rph1* to *Rph15*. MSc thesis. University of Minnesota, United States of America.
- Martin MJ, Chicaiza O, Caffarel JC, Sallam AH, Druka A, Waugh R, Ordon, Kopahnke D, Keilwagen J, Rerovic D, Fetch Jr. TG, Jin Y, Franckowiak JD, Steffenson BJ. 2020. Development of barley introgression lines carrying the leaf rust resistance genes *Rph1* to *Rph15*. *Crop Science* 60:282-302.
- McDonald BA, Linde C. 2002. Pathogen population genetics, evolutionary potential and durable resistance. *Annual Review of Phytopathology* 40:349-379.
- McIntosh RA, Wellings CR, Park RF. 1995. *Wheat Rusts: An Atlas of Resistance Genes*. CSIRO Publications, East Melbourne, Australia.
- McNeal FH, Konzak CF, Smith EP, Tate WS, Russell TS. 1971. A uniform system for recording and processing cereal research data. *Agricultural Research Service Bulletin*. United State Department of Agriculture Washington 34:121-143.
- Mehnaz M, Dracatos P, Pham A, March T, Maurer A, Pillen K, Forrest K, Kulkarni T, Pourkheirandish M, Park RF, Singh D. 2021. Discovery and fine mapping of *Rph28*: a new gene conferring resistance to *Puccinia hordei* from wild barley. *Theoretical and Applied Genetics* 134:2167-2179.
- Melville SC, Griffin GW, Jemmett JL. 1976. Effects of fungicide spraying on brown rust and yield in spring barley. *Plant Pathology* 25:99-107.
- Melville SC, Lanham CA. 1972. Survey of leaf diseases of spring barley in South West England. *Plant Pathology* 21:59-62.
- Morrow CA, Fraser JA. 2009. Sexual reproduction and dimorphism in the pathogenic

## REFERENCES

- basidiomycetes. FEMS Yeast Research 9:161-177.
- Mueller D, Robertson A. 2008. Preventative vs. Curative Fungicides. Integrated Crop Management. [online] Crops.extension.iastate.edu. Available at <https://crops.extension.iastate.edu/cropnews/2008/07/preventative-vs-curative-fungicides> [Accessed 22 May2021].
- Murray TD, Parry DW, Cattlin ND. 1998. A colour handbook of diseases of small grain cereal crops. First edition. United States of America. CRC Press. Iowa State University Press.
- Newton M, Peterson B, Meredith WOS. 1945. The effect of leaf rust of barley on the yield and quality of barley varieties. Canadian Journal of Research 23:212-218.
- Niks RE. 1983. Comparative histology of partial resistance and the non-host reaction of leaf rust pathogens in barley and wheat seedlings. Phytopathology 73:60-64.
- Park RF. 2000. Rust Fungi. Encyclopedia of Microbiology 4:179-195.
- Park RF. 2003. Pathogenic specialization and pathotype distribution of *Puccinia hordei* in Australia, 1992 to 2001. Plant Disease 87:1311-1316.
- Park RF. 2008. Breeding cereals for rust resistance in Australia. Plant Pathology 57:591-602.
- Park RF, Burdon JJ, Jahoor A. 1999. Evidence for somatic hybridisation in nature in *Puccinia recondita* f. sp. *tritici*, the leaf rust pathogen of wheat. Mycological Research 103:715-723.
- Park RF, Golegaonkar PG, Derevnina L, Sandhu KS, Karaoglu H, Elmansour HM, Dracatos PM, Singh D. 2015. Leaf rust of cultivated barley: Pathology and Control. University of Sydney. Annual Review of Phytopathology 53:565-589.
- Park RF, Karakousis A. 2002. Characterization and mapping of gene *Rph19* conferring resistance to *Puccinia hordei* in the cultivar “Reka 1” and several Australian barleys. Plant Breeding 121:232-236.
- Parlevliet JE. 1976. Evaluation of concept of horizontal resistance in barley- *Puccinia hordei* host-pathogen relationship. Phytopathology 66:494-497.

## REFERENCES

- Parlevliet JE. 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 JE, Kievit C. 1986. Development of barley leaf rust, *Puccinia hordei*, infections in barley. Effect of partial resistance and plant stage. *Euphytica* 35:953-959.
- Parlevliet JE, Kuiper HJ. 1977. Resistance of some barley cultivars to leaf rust, *Puccinia hordei* - polygenic, partial resistance hidden by monogenic hypersensitivity. *Netherlands Journal of Plant Pathology* 83:85-89.
- Parlevliet JE, Ommeren 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.
- Paulitz T, Steffenson B. 2011. Biotic stress in barley In: Disease problems and solutions. Ullrich SE. (ed). United States of America, Ames Wiley-Blackwell.
- Peakall R, Smouse PE. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6:288-295.
- Peakall R, Smouse PE. 2012. GENALEX 6.5: Genetic analysis in Excel. Population genetic software for teaching and research - an update. *Bioinformatics* 28:2537-2539.
- Pereyra S, Ackermann MD. 2007. Use of fungicides to control leaf rust of barley and wheat. INIA LA Estanzuela. (Spanish).
- Perrier X, Flori A, Bonnot F. 2003. Data analysis methods. In: Barley improvement, production, and uses. Genetic diversity of cultural tropical plants. Hamon P, Seguin M, Perrier X, Glazmann JC. (eds). United Kingdom. Science Publishers.
- Peterson RF, Campbell AB, Hannah AE. 1948. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. *Canadian Journal of Research* 26:496-500.
- Peterson RH. 1974. The rust fungus life cycle. *Botanical Review* 40:453-513.
- Pickering RA, Malyshev S, Kunzel G, Johnston PA, Korzun V, Menke M, Schubert I. 2000. Locating introgressions of *Hordeum bulbosum* chromatin within the *H. vulgare* genome. *Theoretical and Applied Genetics* 100:27-31.

## REFERENCES

- Pickering RA, Steffenson BJ, Hill AM, Borovkova IG. 1998. Association of leaf rust and powdery mildew resistance in a recombinant derived from a *Hordeum vulgare* × *Hordeum bulbosum* hybrid. *Plant Breeding* 117:83-84.
- Policy RW, Clarkson JDS. 1978. Forecasting cereal disease epidemics. *Infection Plant Disease*. United Kingdom. *Plant Pathology Epidemiology*.
- Pourkheirandish M, Komatsuda T. 2007. The importance of barley genetics and domestication in a global perspective. *Annals of Botany* 100:999-1008.
- Pretorius ZA, Booysen GJ, Boshoff WHP, Joubert JH, Maree GJ, Els J. 2019. Additive manufacturing of devices used for collection and application of cereal rust urediniospores. *Frontiers in Plant Science* 10:639.
- Pretorius ZA, Pakendorf KW, Marais GF, Prins R, Komen JS. 2007. Challenges for sustainable cereal rust control in South Africa. *Australian Journal of Agricultural Research* 58:593-601.
- Pretorius ZA, Park RF, Wellings CR. 2000. An accelerated method for evaluating adult - plant resistance to leaf and stripe rust in spring wheat. *Acta Phytopathologica et Entomologica Hungarica* 35:359-364.
- Pretorius ZA, Visser B, Terefe T, Herselman L, Prins R, Soko T, Siwale J, Mutari B, Seling TI, Hodson DP. 2015. Races of *Puccinia triticina* detected on wheat in Zimbabwe, Zambia and Malawi and regional germplasm responses. *Australasian Plant Pathology* 44:217-224.
- Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945-959.
- Qi X, Niks RE, Stam P, Lindhout P. 1998. Identification of QTLs for partial resistance to leaf rust (*Puccinia hordei*) in barley. *Theoretical and Applied Genetics* 96:1205-1215.
- Reinhold M, Sharp EL. 1982. Resistance to leaf rust of barley in Southern Texas. *Cereal Rusts Bulletin* 10:4-10.
- Riaz A, Periyannan S, Aitken E, Hickey L. 2016. A rapid phenotyping method for adult plant resistance to leaf rust in wheat. *Plant Methods* 12:1-10.

## REFERENCES

- Roane CW. 1972. Barley leaf rust around the world: A review. *Barley Newsletter* 15:23-27.
- Roelfs AP. 1982. Effects of barberry eradication on stem rust in the United States. *Plant Disease* 66:177-181.
- Roelfs AP, Growth JV. 1980. A comparison of virulence phenotypes in wheat stem rust populations reproducing sexually and asexually. *Journal of Molecular Microbiology and Biotechnology* 3:207-214.
- Roelfs AP, Singh RP, Saari EE. 1992. *Rust diseases of wheat: Concepts and methods of disease management*. Mexico. CIMMYT.
- Rothwell CT, Singh D, Dracatos PM, Park RF. 2020. Inheritance and characterization of *Rph27*: a third race-specific resistance gene in the barley cultivar Quinn. *Phytopathology* 110:1067-1073.
- Rothwell CT, Singh D, Van Ogtrop F, Sorensen C, Fowler R, Park RF, Dracatos P. 2019. Rapid phenotyping of adult plant resistance in barley (*Hordeum vulgare*) to leaf rust under controlled conditions. *Plant Breeding* 198:51-61.
- Saghai-Marooif MA, Soliman KM, Jorgensen RA, Allard RW. 1984. Ribosomal DNA spacer-length polymorphisms in barley. Mendelian inheritance, chromosomal location and population dynamics. *Proceedings of the National Academy Science USA* 81:8014–8018.
- SAGIS. 2021. South African Grain Information Service. Available at <https://www.sagis.org.za>. [Accessed 05 April 2021].
- Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular cloning, a laboratory manual*. Second edition. New York. Cold Spring Harbor Laboratory Press.
- Sandhu KS. 2011. Genetic and molecular analyses of barley for seedling and adult plant resistance against rust diseases. Doctor of Philosophy Thesis, Plant Breeding Institute Cobbitty University of Sydney, Australia.
- Sandhu KS, Forrest KL, Kong S, Bansal UK, Singh D. 2012. Inheritance and molecular mapping of a gene conferring seedling resistance against *Puccinia hordei* in the barley cultivar Ricardo. *Theoretical and Applied Genetics* 125:111-143.

## REFERENCES

- Sandhu KS, Karaoglu H, Park RF. 2016. Pathogenic and genetic diversity in *Puccinia hordei* Otth in Australasia. *Journal of Plant Breeding and Crop Science* 8:197-205.
- Schneider S, Excoffier L, Laval G. 2010. Arlequin (version 3.5.1.2): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* 1:47-50.
- Scholes JD, Farrar JF. 1987. Development of symptoms of brown rust of barley in relation to the distribution of fungal mycelium, starch accumulation and localised changes in the concentration of chlorophyll. *New Phytologist* 107:103-117.
- Semeane Y, Hundie B, Woldeab G, Tadesse D. 1996. Disease survey and loss assessment studies on barley. In: *Barley Research in Ethiopia: Past Work and Future Prospects*. Gebre H, Van Leur J. (eds). Ethiopia. *International Academic Research Journal*. 105-115.
- Simkin MB, Wheeler BEJ. 1974. The development of *Puccinia hordei* on barley cv. Zephyr. *Annals of Applied Biology* 78:225-235.
- Singh D, Dracatos P, Derevnina L, Zhou MX, Park RF. 2015. *Rph23*: A new designated additive adult plant resistance gene to leaf rust in barley on chromosome 7H. *Plant Breeding* 134:62-69.
- Singh D, Macaigne N, Park RF. 2013. *Rph20*: Adult plant resistance gene to barley leaf rust can be detected at early growth stages. *European Journal of Plant Pathology* 137:719-725.
- Singh D, Park RF, McIntosh RA. 2001. Inheritance of seedling and adult plant resistance to leaf rust of selected Australian spring and English winter wheat cultivars. *Plant Breeding* 120:503-507.
- Singh RP, Gupta AK. 1992. Expression of wheat leaf resistance gene *Lr34* in seedlings and adult plants. *Plant Disease* 76:489-491.
- Singh RP, Hodson D, Huerta-Espino J, Jin Y, Bhavani S, Njau P, Herrera-Foessel S, Singh PK, Singh S, Govindan V. 2011. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. *Annual Review of Phytopathology* 49:465-481.

## REFERENCES

- Smit G, Parlevliet JE. 1990. Mature plant - resistance of barley to barley leaf rust, another type of resistance. *Euphytica* 50:159-162.
- Stakman EC, Piemeisel FJ. 1917. Biologic forms of *Puccinia graminis* on cereals and grasses. *Journal of Agricultural Research* 10:429-495.
- Stakman EC, Stewart DM, Loegering WQ. 1962. Identification of physiologic races of *Puccinia graminis* var. *tritici*. Agricultural Research Service E617. Washington DC: USDA.
- STATISTA. 2021. Available at <http://www.statista.com>. [Accessed 05 April 2021].
- Stenberg P, Lundmark M, Saura A. 2003. MLGsim: A program for detecting clones using a simulation approach. *Molecular Ecology Notes* 3:329-331.
- Stevenson JA, Johnson AG. 1946. The nomenclature of the barley leaf rust. *Plant Disease Report* 30:372.
- Sun Y, Neate S. 2007. Genetics and molecular mapping of *Rph13*, a gene conferring resistance to leaf rust in barley. *Phytopathology* 97:S112.
- Tan BH. 1977. New gene for resistance to *Puccinia hordei* in certain Ethiopian barleys. *Cereal Rusts Bulletin* 5:39-43.
- Tan BH. 1978. Verifying the genetic relationships between three leaf rust resistance genes in barley. *Euphytica* 27:317-323.
- Teng PS, Close RC. 1980. Effect of solar radiation on survival of *Puccinia hordei* urediniospores in New Zealand. *Cereal Rust Bulletin* 8:23-29.
- Terefe T, Visser B, Herselman L, Selinga T, Pretorius ZA. 2014. First report of *Puccinia triticina* (leaf rust) race FBPT wheat in South Africa. *Plant Disease* 98:1001.
- Toome M, Aime MC. 2012. "Pucciniomycetes." The Tree of Life Web Project Retrieved 2020. Available at <http://tolweb.org/Pucciniomycetes/51246>.
- Tranzschel W. 1914. Kulturversuche mit uredineen in den Jahren 1911–1913. *Vorlauf Mitt. Mycology* 4:70-71.
- Tucker MA, Jayasena K, Ellwood SR, Oliver RP. 2013. Pathotype variation of barley powdery

## REFERENCES

- mildew in Western Australia. *Australasian Plant Pathology* 42:617-623.
- Tuleen NA, McDaniel ME. 1971. Location of genes *Pa* and *Pa5*. *Barley Newsletter* 15:106-107.
- Van Niekerk BD, Pretorius ZA, Boshoff WHP. 2001a. Occurrence and pathogenicity of *Puccinia hordei* on barley in South Africa. *Plant Disease* 85:713-717.
- Van Niekerk BD, Pretorius ZA, Boshoff WHP. 2001b. Potential yield losses caused by barley leaf rust and oat leaf and stem rust to South African barley and oat cultivars. *South African Journal of Plant and Soil* 18:108-113.
- Visser B, Herselman L, Bender CM, Pretorius ZA. 2012. Microsatellite analysis of selected *Puccinia triticina* races in South Africa. *Australian Plant Pathology* 41:165-171.
- Visser B, Herselman L, Pretorius ZA. 2009. Genetic comparison of Ug99 with selected South African races of *P. graminis* f. sp. *tritici*. *Molecular Plant Pathology* 10:213-222.
- Visser B, Herselman L, Pretorius ZA. 2016. Microsatellite characterisation of South African *Puccinia striiformis* races. *South African Journal of Plant and Soil* 33:161-166.
- Visser B, Meyer M, Park RF, Gilligan CA, Burgin LE, Hort MC, Hodson DP, Pretorius ZA. 2019. Microsatellite analysis and urediniospore dispersal simulations support the movement of *Puccinia graminis* f. sp. *tritici* from southern Africa to Australia. *Phytopathology* 109:133-144.
- Von Bothmer R, Komatsuda T. 2011. Barley origin and related species: In *Barley Production, Improvement and Uses*. Ullrich SE. (ed). Chichester: Wiley-Blackwell Press.
- Vos P, Hogers R, Bleeker M, Reijans M, Van De Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M. 1995. AFLP; a new technique for DNA fingerprinting. *Nucleic Acids Research* 23:4407-4414.
- Wallwork H, Butt M, Capio E. 2016. Pathogen diversity and screening for minor gene resistance to *Pyrenophora teres* f. sp. *teres* in barley and its use for plant breeding. *Australasian Plant Pathology* 45:527-531.
- Wallwork H, Preece P, Cotterill PJ. 1992. *Puccinia hordei* on barley and *Ornithogalum umbellatum* in South Australia. *Australian Plant Pathology* 21:95-97.

## REFERENCES

- Walters D, Avrova A, Bingham I, Burnett F, Fountaine J, Havis N, Hoad S, Hughes G, Looseley M, Oxley S, Renwick A, Topp C, Newton A. 2012. Control of foliar diseases in barley: towards an integrated approach. *European Journal of Plant Pathology* 133:33-73.
- Wang L, Wang Y, Wang Z, Marcel TC, Niks RF, Qi X. 2010. The phenotypic expression of QTLs for partial resistance to barley leaf rust during plant development. *Theoretical and Applied Genetics* 121:857-865.
- Waterhouse WL. 1927. Studies in the inheritance of resistance to leaf rust *Puccinia anomala* Rostr. in crosses of barley. *Journal and Proceedings of the Royal Society of New South Wales* 61:218-247.
- Weerasena JS, Steffenson BJ, Falk AB. 2004. Conversion of an amplified fragment length polymorphism marker into a co-dominant marker in the mapping of the *Rph15* gene conferring resistance to barley leaf rust, *Puccinia hordei* Otth. *Theoretical and Applied Genetics* 108:712-719.
- Whelan HG, Gaunt RE, Scott WR. 1997. The effect of leaf rust (*Puccinia hordei*) on yield response in barley (*Hordeum vulgare* L.) crops with different yield potentials. *Plant Pathology* 46:397-406.
- Wicker T, Krattinger SG, Lagudah ES, Komatsuda T, Pourkheirandish M, Matsumoto T, Cloutier S, Reiser L, Kanamori H, Sato K, Perovic D, Stein N, Keller B. 2009. Analysis of intraspecies diversity in wheat and barley genomes identifies breakpoints of ancient haplotypes and provides insight into the structure of diploid and hexaploid triticeae gene pools. *Plant Physiology* 149:258-270.
- Winter P, Kahal G. 1995. Molecular marker technologies for plant improvement. *World Journal of Microbiology and Biotechnology* 11:438-448.
- Yu X, Kong HY, Meiyalaghan V, Casonato S, Cheng S, Jones EE, Butler RC, Pickering R, Johnston PA. 2018. Genetic mapping of a barley leaf rust resistance gene *Rph26* introgressed from *Hordeum bulbosum*. *Theoretical and Applied Genetics* 131:2567-2580.
- Zadoks JC, Chang TT, Konzak CF. 1974. A decimal code for the growth stages of cereals. *Weed Research* 14:415-421.

## REFERENCES

- Zhong S, Effertz RJ, Jin Y, Franckowiak JD, Steffenson BJ. 2003. Molecular mapping of the leaf rust resistance gene *Rph6* in barley and its linkage relationships with *Rph5* and *Rph7*. *Phytopathology* 93:604-609.
- Ziems L, Hickey LT, Platz GJ, Franckowiak JD, Dracatos PM, Singh D, Park RF. 2017. Characterisation of *Rph24*: A gene conferring adult plant resistance to *Puccinia hordei* in barley. *Phytopathology* 107:1-30.
- Zillinsky FJ. 1983. Common diseases of small grain cereals. A guide to identification. Mexico. CIMMYT.

**Appendix 1** List of *Puccinia hordei* isolates included in the study.

Isolate/race	Locality	Coordinates	Origin of sample	DNA extraction
UVPh3231	UVIs_44_ARC-SG	Unknown	Historic (1994)	UVPh3231
UVPh7231	UVIs_52_ARC-SG	Unknown	Historic (1998)	UVPh7231
Ph3_Gt2015	Greytown	S29°08'16.1" E30°37'10.8"	Barley trial plots	UVPh7235
Ph1_Rs2017	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	Ph1_Rs2017
Ph2_Rs2017	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	Ph2_Rs2017
Ph3_Rs2017	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	Ph3_Rs2017
Ph4_Rs2017	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	Ph4_Rs2017
Ph5_Rs2017	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	Ph5_Rs2017
Ph6_Rs2017	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	Ph6_Rs2017
Ph7_Rs2017	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	Ph7_Rs2017
Ph1.1_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	
Ph1.2_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph1.2_Np2018
Ph2.1_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph2.1_Np2018
Ph2.2_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	
Ph3.1_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph3.1_Np2018
Ph3.2_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	
Ph4.1_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph4.1_Np2018
Ph4.2_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph4.2_Np2018
Ph5.1_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph5.1_Np2018
Ph5.2_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph5.2_Np2018
Ph6.1_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph6.1_Np2018
Ph6.2_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	
Ph7.1_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph7.1_Np2018
Ph7.2_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	
Ph8.1_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph8.1_Np2018
Ph8.2_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	
Ph9.1_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph9.1_Np2018

## APPENDIX

**Appendix 1** (cont) List of *Puccinia hordei* isolates included in the study.

Isolate/race	Locality	Coordinates	Origin of sample	DNA extraction
Ph9.2_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	
Ph10.1_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph10.1_Np2018
Ph10.2_Np2018	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	
Ph11.1_Rs2018	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	Ph11.1_Rs2018
Ph11.2_Rs2018	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	Ph11.2_Rs2018
Ph12.1_Rs2018	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	Ph12.1_Rs2018
Ph12.2_Rs2018	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	
Ph13.1_Rs2018	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	Ph13.1_Rs2018
Ph13.2_Rs2018	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	
Ph14.1_Rs2018	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	Ph14.1_Rs2018
Ph14.2_Rs2018	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	
Ph15.1_Rs2018	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	Ph15.1_Rs2018
Ph15.2_Rs2018	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	
Ph16.1_Rs2018	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	Ph16.1_Rs2018
Ph16.2_Rs2018	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	Ph16.1_Rs2018
Ph1.1_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph1.1_Np2019
Ph1.2_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	
Ph2.1_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph2.1_Np2019
Ph2.2_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	
Ph2.3_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	
Ph3.1_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph3.1_Np2019
Ph3.2_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	
Ph4.1_HA2019	Hemel and Aarde Valley	S34°23'05.6" E19°14'03.4"	Farmers field	Ph4.1_HA2019
Ph4.2_HA2019	Hemel and Aarde Valley	S34°23'05.6" E19°14'03.4"	Farmers field	
Ph5.1_HA2019	Hemel and Aarde Valley	S34°23'05.6" E19°14'03.4"	Farmers field	Ph5.1_HA2019
Ph5.2_HA2019	Hemel and Aarde Valley	S34°23'05.6" E19°14'03.4"	Farmers field	Ph5.2_HA2019
Ph6.1_CI2019	Caledon	S34°22'75.7"E19°53'72.2"	Farmers field	Ph6.1_CI2019

## APPENDIX

**Appendix 1** (cont) List of *Puccinia hordei* isolates included in the study.

Isolate/race	Locality	Coordinates	Origin of sample	DNA extraction
Ph6.2_CI2019	Caledon	S34°22'75.7"E19°53'72.2"	Farmers field	
Ph7.1_CI2019	Caledon	S34°22'75.7"E19°53'72.2"	Farmers field	Ph7.1_CI2019
Ph7.2_CI2019	Caledon	S34°22'75.7"E19°53'72.2"	Farmers field	
Ph8.1_Kp2019	Klipdale	S34°24'92.8" E19°97'73.3"	Farmers field	Ph8.1_Kp2019
Ph8.2_Kp2019	Klipdale	S34°24'92.8" E19°97'73.3"	Farmers field	
Ph9.1_Vk2019	Voorstekop	S34°09'62.4" E21°09.27.7"	Barley trial plots	Ph9.1_Vk2019
Ph9.2_Vk2019	Voorstekop	S34°09'62.4" E21°09.27.7"	Barley trial plots	
Ph9.3_Vk2019	Voorstekop	S34°09'62.4" E21°09.27.7"	Barley trial plots	
Ph10.1_CI2019	Caledon	S34°22'75.7" E19°53'72.2"	Farmers field	Ph10.1_CI2019
Ph10.2_CI2019	Caledon	S34°22'75.7" E19°53'72.2"	Farmers field	
Ph11.1_Rs2019	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	Ph11.1_Rs2019
Ph11.2_Rs2019	Riviersonderend	S34°15'77.3" E19°90'86.0"	Barley trial plots	
Ph12.1_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph12.1_Np2019
Ph13.1_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph13.1_Np2019
Ph13.2_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	
Ph14.1_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph14.1_Np2019
Ph14.2_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	
Ph15.1_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph15.1_Np2019
Ph15.2_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	
Ph16.1_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph16.1_Np2019
Ph16.2_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	
Ph17.1_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	Ph17.1_Np2019
Ph17.2_Np2019	Napier	S34°28'37.3" E19°54'17.7"	Barley trial plots	