academicJournals

Vol. 12(42), pp. 6068-6075, 16 October, 2013
DOI: 10.5897/AJB12.989
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Molecular and genetic study of wheat rusts

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Accepted 2 August, 2013

***Puccinia triticina, Puccinia graminis* and *Puccinia striiformis* cause leaf, stem and yellow rust, respectively. Wheat rusts can cause losses as high as 70%. The rusts ability to evolve fungicide resistance has resulted in the use of resistant cultivars as the primary method of control. Breeding resistant cultivars is a long process and requires an accurate picture of the current and future pathogen population. Differentiation of wheat rust pathotypes using conventional plant pathology techniques is time consuming, labour intensive and requires the services of a highly skilled and experienced plant pathologist. Modern molecular biology techniques have the potential to aid the conventional techniques and provide fast, accurate same-day results. Microsatellite markers were used to differentiate *P. triticina* and *P. striiformis* pathotypes. Amplified fragment length polymorphisms (AFLP) were used to differentiate stem rust *P. graminis* pathotypes. Phylogenetic trees were created for leaf and stem rust pathotypes. Field isolates of leaf, stem and yellow rust were collected from eleven sites across the Western Cape Province. Microsatellite markers were used to type leaf and yellow rust isolates. AFLP markers could not be used on field isolates due to the presence of plant DNA. Novel alleles found in the Leaf and yellow rusts isolates prevented the assigning of a specific pathotype to each isolate. UVPrt10 (25.2%) and UVPrt9 (21.5%) were the most prevalent leaf rust pathotypes. Only 6E16A+ was identified in the yellow rust isolates. Pathotype incidence was similar to previous studies. The prevalence of multiple pathotypes with a variety of virulence genes in the rust population shows that breeding lines with single major resistance genes will not be effective and breeding programmes should concentrate on lines that exhibit quantitative resistance.**

Key words: Prevalence, microsatellite, amplified fragment length polymorphisms (AFLP), phylogeny, *Puccinia*.

INTRODUCTION

Puccinia triticina Eriks, *Puccinia graminis* f.sp. *tritici* Eriks and Henn and *Puccinia striiformis* Westend f.sp. *tritici* Eriks are the causative agents of leaf, stem and yellow wheat rust, respectively. Wheat rusts are one of the primary biotic restrictors of wheat production globally (Keiper et al., 2006). Rust infections can cause losses as high as 70% (Murray et al., 1998).

The rusts are highly adaptable and can rapidly evolve resistance to control methods such as fungicides (Boshoff et al., 2003). Selection is the most powerful force shaping the genetic diversity of a population (McDonald and Linde, 2002). In wheat, rust selection is caused by the widespread introduction of resistant cultivars tha cause, cause an increase in the frequency of virulent alleles (Harvey et al., 2001).

The effect of selection can be reduced by several methods. Pyramiding several major resistance genes and breeding for durable resistance based on the amassing of additive minor genes through nonspecific pathotype (slow rusting) resistance is a successful strategy. With slow rusting, disease progression is not prevented but rather slowed. The result is intermediate to low levels of disease with all the pathotypes of the particular pathogen (Duvellier et al., 2007; Li et al. 2010). Such quantitative resistance

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cannot be rapidly broken and will only be gradually eroded (McDonald and Linde, 2002).

Rotation of genes, that is, where different major resistance genes are rotated in time and space or cultivars with different combinations of resistance genes are grown; has also been shown to disrupt selection (Zhu et al., 2000; McDonald and Linde, 2002).

In South Africa, rust control has focused on the introduction of resistant cultivars which has been shown to be an effective method of reducing rust infections (Pretorius et al., 2007). However, the use of these resistant cultivars must be carefully managed because if they are introduced and used too widely (effectively creating a monoculture) the rusts will quickly develop new virulences due to the very high selective pressures being placed upon them (McDonald and Linde, 2002) as can be seen with the emergence of race TTKS in East Africa in 1999 (Pretorius et al., 2000; Expert Panel on the Stem Rust outbreak in East Africa, 2005) and its rapid spread and acquisition of new virulences (Terefe et al., 2011; Pretorius et al., 2012). Breeding a new resistant cultivar requires extremely long timeframes; sometimes above of fifteen years can pass between the initial cross made for a cultivar and the first release of commercial seed. For this reason it is necessary to have an accurate picture of the pathotype composition of the current and future rust population. In order to gain this knowledge and make predictions as to the future pathogen population structure, population genetic studies of the wheat rusts have to be conducted.

Conventional plant pathology that is used to differentiate the pathotypes of a pathogen, such as the *Puccinia* spp. Is a complex and time consuming process. They require a highly skilled plant pathologist to ensure accurate results. Modern molecular biology techniques have the potential to complement the conventional techniques and generate accurate results the same day (Brown, 1996; McCartney et al., 2003).

A wide variety of molecular markers have been used in the differentiation of wheat fungal pathogens. These include: microsatellite markers (Enjalbert et al., 2002; Szabo and Kolmer, 2007; Szabo, 2007; Zhong et al., 2009; Kolmer et al., 2013; Kolmer, 2013), restriction fragment length polymorphisms (Chen et al., 1994; Keller et al., 1997a, b; Zhan et al., 1998), Southern Blots (Shan et al., 1998), random amplification of polymorphic differences (Kolmer et al., 1994; Park et al., 2000), amplified fragment length polymorphism (AFLP) (Visser et al., 2009), and high resolution melt analysis of Real-time polymerrase chain reaction (RT-PCR) of the ITS region of the rDNA gene (Schena et al., 2004; Barnes and Szabo, 2007; Liu et al., in press) and whole genome sequencing (Cuomo et al., 2010).

There are several genetic and practical considerations that affect a particular marker systems efficacy in differentiating between the pathotypes of a pathogen (Brown, 1996). Microsatellite markers satisfy most of the conditions as they are highly polymorphic, co-dominant and relatively easy to score. They are also suitable for use in high throughput systems. AFLP markers also meet many of the conditions.

Molecular marker based techniques have demonstrated that they possess the potential to complement conventional plant pathology techniques in the differentiation of fungal pathogens of wheat.

MATERIALS AND METHODS

The following rust pathotypes were used: Stem rust: UVPgt50 (2SA4), UVPgt51 (2SA36), UVPgt52 (2SA100), UVPgt53 (2SA102), UVPgt54 (2SA55), UVPgt55 (2SA88), UVPgt56 (2SA102K) and UVPgt57 (2SA105). Leaf rust: UVPrt2, UVPrt3 (3SA123), UVPrt4, UVPrt5, UVPrt8 (3SA132), UVPrt9 (3SA133), UVPrt10 (3SA126), UVPrt13 (3SA140) and UVPrt19. Yellow rust: 6E16A-, 6E22A- and 7E22A- All the samples were obtained as frozen urediniospores from L. Snyman (University of Stellenbosch). The original source of most of the samples was Prof. Z. Pretorius (University of the Free State).

DNA extractions were done from 40 µg of frozen urediniospores. The CTAB protocol of Liu and Kolmer (1998) was followed. Primers: All primers were used at a concentration of 10 ng/µl and were obtained from IDT. All PCRs were performed in an Applied Biosystems 2720 Thermal Cycler.

Microsatellites

The reaction mix to amplify the leaf and yellow rust microsatellites was as follows: 1 µl 10X Kapa Buffer A (KapaBiosystems), 2 µl 25 mM $MgCl_2$ (KapaBiosystems), 0.8 µl 10 mM Kapa dNTP mix, a volume of primer as specified in Table 1, 0.042 µl 5 U/µl KapaTaq (KapaBiosystems), 1 µg 100 ng/µl DNA and sufficient distilled, autoclaved water (dH_2O) to make the final reaction volume 10 µl. The following PCR programme was used to amplify the microsatellites: 2 min at 95°C followed by 35 cycles of 30 s at 95°C, 30 s at 60°C, and 30 s at 72°C with a final elongation step of 10 min at 72°C. The reaction products were analysed on an Applied Biosystems 3130xl Genetic Analyser. The electropherograms were analysed using GeneMapper 4.0 (Applied Biosystems).

AFLP

AFLP primers from Visser et al. (2009) were used to differentiate the stem rust pathotypes (Table 2). The AFLP protocol from Honing (2007) was used. The reaction products were analysed on an Applied Biosystems 3130xl Genetic Analyser. The electropherograms were analysed using GeneMapper 4.0 (Applied Biosystems).

Microsatellite data analysis: A matrix that recorded the size of each specific microsatellite fragment for each pathotype was constructed in Excel. Data analysis was performed using Power Marker v3.25 (Liu and Muse, 2005; http://statgen.ncsu.edu/powermarker/). PowerMarker was chosen because of its ability to handle microsatellite data as well as complete all the required calculations with no additional software required. The data was imported into PowerMarker from a text file. The allele frequencies were calculated. Genetic distances were calculated from the frequency data using the CS Cord 1967 distance calculation (Cavalli-Sforza and Edwards, 1967) as it has been shown that this model can produce true tree topology irrespective of the microsatellite mutation model used (Takeszaki and Nei, 1996). Cladograms were generated in PowerMarker using the neighbour-Joining clustering method, as this method is more suited to determine tree topology from CS Cord distance than the unweighted pair group method with arithmetic mean (UPGMA) method (Takeszaki and Nei, 1996). Cladograms

Table 1. Primers used to type leaf and yellow rust field isolates.

Name	Label	Sequence	Volume (µl)	Annealing temperature (°C)
Leaf rust				
PtSSR68-F	NED	5' – GAC TCA GCC CAC TGC TAA CC- 3'	0.250	60
PtSSR68-R		5' – GAT GGC GAC GTA TTT GGT CT- 3'	0.250	60
PtSSR151A-F	VIC	5' – TCA TCG CAC TCC ACT CAG AC- 3'	0.225	60
PtSSR151A-R		5' – ATG CTG CCC AAC CTG CTC- 3'	0.225	60
PtSSR154-F	NED	5' – ACG GTC AAC AGC CAA CTA CC- 3'	0.225	60
PtSSR154-R		5' – CCT CGT CAT CCT GGT TGA GT- 3'	0.225	60
Yellow rust				
RJ22-F	6-FAM	5' - CCC TTC GTC TGT CAT CCG - 3'	0.350	60
RJ22-R		5' - ATC AAG AAG ATT CCT GGG TGA G - 3'	0.350	60
RJ27-F	NED	5' - CGT CCC GAC TAA TCT GGT CC - 3'	0.300	60
RJ27-R		5' - ATG AGT TAG TTT AGA TCA GGT CGA C - 3'	0.300	60

Table 2. Sequences of the AFLP primers used to differentiate the pathotypes of stem rust.

Name	Label	Sequence	Volume (µl)
EcoRI Adapter[a]		5'- CTC GTA GAC TGC GTA CC -3' 3'- CAT CTG ACG CAT GGT TAA -5'	1.00
MseI Adapter[a]		5'- GAC GAT GAG TCC TGA G -3' 3'- TAC TCA GGA CTC AT -5'	1.00
EcoRI Primer+0		5'- GAC TGC GTA CCA ATT C -3'	1.50
EcoRI Primer+1.0	NED	5'- GAC TGC GTA CCA ATT CA -3'	0.25
EcoRI Primer+2.1	VIC	5'- GAC TGC GTA CCA ATT CAA -3'	0.25
EcoRI Primer+2.2	6-FAM	5'- GAC TGC GTA CCA ATT CCC -3'	0.75
EcoRI Primer+2.3	PET	5'- GAC TGC GTA CCA ATT CTG -3'	0.75
MseI Primer+0		5'- GAT GAG TCC TGA GTA A -3'	1.50
MseI Primer+2.1		5'- GAT GAG TCC TGA GTA AAT -3'	1.00
MseI Primer+2.2		5'- GAT GAG TCC TGA GTA AAG -3'	1.00
MseI Primer+3.0		5'- GAT GAG TCC TGA GTA ACA T -3'	1.00

[a]Adapters were obtained as double stranded molecules.

were visualized in TreeView v1.66 (http://taxonomy.zoology.gla.ac.uk/rod/treeview.html).

AFLP data analysis

A binary matrix for each AFLP fragment was constructed in Excel. The data was then analysed in Power Marker 3.25. The allele frequencies were calculated and the genetic distances were determined using the CS Cord 1967 distance calculation. Dendrograms were generated using the neighbour-joining clustering method. The cladograms were visualised in Tree View 1.66.

Field isolates of leaf and yellow rust were collected from 11 sites in the Overberg and Swartland regions of the Western Cape. The field isolates were collected from wheat and triticale commercial cultivars as well as Stellenbosch University Plant Breeding Laboratory advanced breeding lines. DNA was extracted from the isolates using the protocol from Liu and Kolmer (1998) and diluted to 100 ng/µl.

The leaf and yellow rust isolates were typed using microsatellites markers (Table 1) amplified by fluorescently labelled primers (Szabo and Kolmer, 2007; Enjalbert et al., 2002). Amplification of leaf and yellow rust was done by separate multiplex reactions with a reaction mixture as follows: 1.0 µl 10x Kapa Buffer A (KapaBiosystems), 2.0 µl 25 mM $MgCl_2$ (KapaBiosystems), 0.8 µl 10 mM Kapa dNTP mix (KapaBiosystems), a volume of primer as specified in Table 1, 0.042 µl 5 U/µl KapaTaq (KapaBiosystems), 1.0 µl DNA, and sufficient distilled, autoclaved water (dH_2O) to make the final volume 10 µl. The PCR programme used to amplify the microsatellites was 2 min at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 60°C, 30 s at 72°C, concluding with 10 min at 72°C and a soak temperature of 4°C.

RESULTS

Eighteen microsatellite primer pairs (Szabo and Kolmer, 2007) were used for leaf rust. Of the eighteen, eight pairs either did not amplify or were monomorphic across all the pathotypes. The remaining ten primers pairs amplified twenty nine alleles (including six null alleles) across the

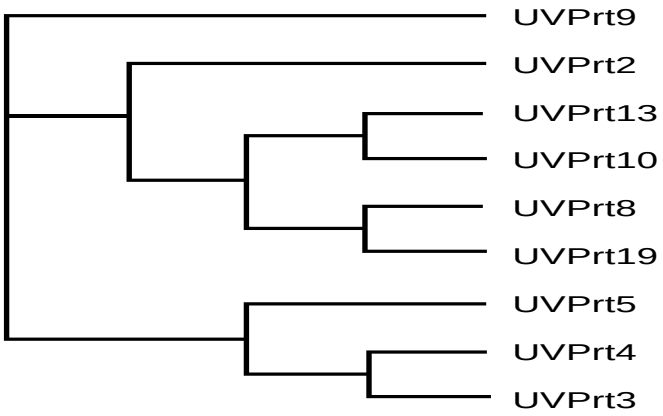

Figure 1. Cladogram of leaf rust pathotypes based on microsatellite data.

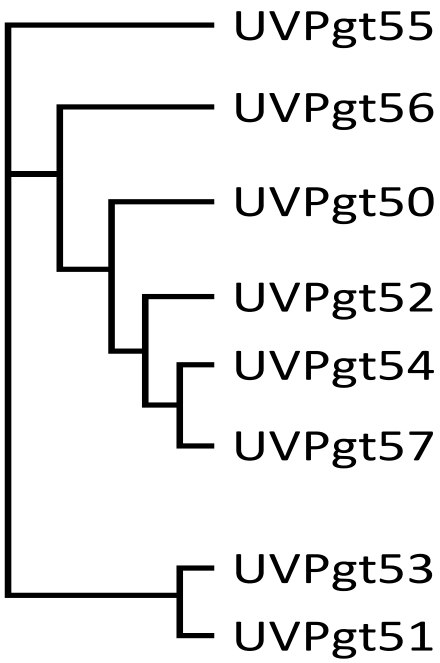

Figure 2. Cladogram of stem rust pathotypes based on AFLP data.

pathotypes. A cladogram was constructed (Figure 1) based on the genetic distances between the pathotypes. The analysis grouped the pathotypes into three main groups with UVPrt 2, UVPrt 13, UVPrt 10, UVPrt 8 and UVPrt 19 together; UVPrt 5, UVPrt 4, UVPrt 3 together and UVPrt 9 alone. It was possible to distinguish between the pathotypes using a subset of the microsatellites, namely PtSSR68, PtSSR151A and PtSSR154.

Stem rust samples had insufficient diversity to allow the pathotypes to be distinguished using only the microsatellites from Szabo (2007) and Zhong et al. (2009).

Twelve different AFLP primer combinations were used, yielding 1926 reproducible fragments. A cladogram was constructed based on the genetic distances between the pathotypes (Figure 2). There were three main groups, UVPgt55 on its own, UVPgt50, UVPgt52, UVPgt54, UVPgt56 and UVPgt57 in the second group and UVPgt51 and UVPgt53 in the third group. It was not possible to distinguish between the pathotypes using only the microsatellite markers as there was insufficient allelic diversity found.

Twelve microsatellite primer pairs were obtained from Enjalbert et al. (2002) for yellow rust. Of the twelve, nine did not amplify or were monomorphic across all the pathotypes. The other three primers (RJ3, RJ22 and RJ27) amplified nine alleles (including three null alleles) across the pathotypes. It was possible to differentiate between the pathotypes using only two markers, RJ22 and RJ27.

Ninety one field isolates of rust were collected from eleven sites in the Swartland and Overberg regions of the Western Cape (Figures 1 and 2). Of these, fifty six were leaf rust, thirty seven were stem rust and three were yellow rust. Many of the isolates were infected by multiple pathotypes and multiple species of wheat rust. Novel alleles were found in the leaf and yellow rust isolates which when combined with the presence of multiple pathotypes and species in some isolates precluded the unambiguous assigning of a specific pathotype or pathotypes to each isolate.

DISCUSSION

Szabo and Kolmer (2007) as well as Kolmer et al., 2013 and Kolmer (2013) found much more diversity at the microsatellite loci tested in their leaf rust samples than was found in this study. These observed differences could be due to only one sample per pathotype being tested or limited diversity in leaf rust in South Africa. The cited studies tested leaf rust isolated from far larger areas than this study which could account for the observed differences. The phylogenetic analysis of the leaf rust pathotypes created three separate groupings: UVPrt9 alone; UVPrt2, UVPrt13, UVPrt10, UVPrt8 and UVPrt19; and UVPrt5, UVPrt4 and UVPrt3. The positioning of UVPrt9 in a separate group to the other pathotypes would imply that it was introduced into South Africa relatively recently, whereas the other two groups appear to have been present in South Africa for some time, allowing differentiation to occur.

Due to the lack of diversity at the tested microsatellite loci, replicating the findings of Visser et al. (2009) and contrary to that of Szabo (2007) and Zhong et al. (2009), it was not possible to distinguish the pathotypes of stem rust. The lack of diversity is probably due to reasons similar to those outlined above for leaf rust. AFLP markers were used, and were found to be highly diverse, again replicating the results of Visser et al. (2009). There was sufficient diversity in the AFLP markers to distinguish each pathotype. This study found significantly more AFLP fragments than Visser et al. (2009) but this can be attribu-

buted to the use of fluorescently labelled primers and the analysis on the automated sequencer, which is much more sensitive than the analysis of poly-acrylamide gels. The phylogenetic analysis of the AFLP data created three separate groups: UVPgt55 alone; UVPgt56, UVPgt50, UVPgt52, UVPgt57 and UVPgt54; and UVPgt53 and UVPgt51 (Figure 2). On an avirulence-virulence level UVPgt55 is very closely related to the East African rust pathotype Ug99 (Pretorius et al., 2007). The positioning of UVPgt55 separately from the other pathotypes and its close pathogenic relationship to Ug99 supports the conclusion that UVPgt55 did not originate in South Africa but was introduced. Visser et al. (2009) used AFLP markers to compare South African stem rust pathotypes to Ug99 and came to the same conclusion. AFLP markers are not suitable for the analysis of field samples as the process of amplifying the DNA is not specific to fungal DNA and will be contaminated by the presence of plant DNA. Individual AFLP markers, specific to each pathotype, could be sequenced and specific primers designed allowing the analysis of field isolates. Our inability to type field isolates of stem rust meant that we could draw no comparisons with the findings of Terefe et al. (2010).

A study by Enjalbert et al. (2002), of ninety six yellow rust isolates from France and China found low levels of diversity at the loci tested. As yellow rust has only beeen recorded in South Africa since 1996 (Pretorius et al., 1997) the expectation was that even lower levels of diversity would be found as South African Yellow rust has not had sufficient time to diversify to the same extent as the yellow rust found internationally. There was sufficient polymorphism in the loci tested (nine alleles across all pathotypes) to allow the pathotypes to be differentiated.

This study found two alleles per locus at the RJ3, RJ17 and RJ21 loci as was found by Enjalbert et al. (2002) and addtionally, seven novel alleles were found, two at the RJ3 locus and one at each of the RJ17, RJ20, RJ22, RJ24 and RJ27 loci. Enjalbert et al. (2002) tested the 6E16 pathotype but do not give the haplotypes for the pathotypes they tested, so it is not possible to compare the South African 6E16 pathotype with the French 6E16 pathotype.

The South African pathotypes are less diverse than the international samples tested by Enjalbert et al. (2002), but this was expected due to the lack of time for differentiation to occur. The novel alleles seems to indicate that the origin of the South African rust pathotypes is not France or China but it is not possible to say this definetively as Enjalbert et al. (2002), do not give the haplotypes of the pathotypes they tested.

Only two primers, RJ22 and RJ27, are required to distinguish between the three yellow rust pathotypes. Phylogenetic analysis of the pathotypes was not done as there are too few pathotypes for meaningful results.

Considerably, more marker diversity was found in the leaf and yellow rust field isolates than had been found in stored samples. However, while these alleles are novel in a South African context, they have already been recorded by Szabo and Kolmer (2007) and Enjalbert et al. (2002). Even with this increase in the number of alleles, South African rust pathotypes still do not display as much diversity as their foreign counterparts. The presence of multiple infections in a single isolate and the presence of novel alleles precluded the definitive assigning of a specific pathotype or pathotypes to each isolate. It also prevented the determination of genotype frequencies which ultimately prevents testing for Hardy-Weinberg equilibrium. Our inability to test for Hardy-Weinberg equilibrium prevents the further use of these markers in population genetic studies until when it is possible to calculate both allele and genotype frequencies.

Leaf rust was found at almost all the eleven localities, this result agrees with the findings of Pretorius et al. (2007), who found leaf rust was widely distributed across the Western Cape (Figures 3 and 4). Yellow rust was confined to only three of the localities (Figures 3 and 4). Yellow rust has a smaller range of optimal growth conditions than leaf rust and the central region of the south Western Cape was the only area in which these conditions occurred during the sampling period.

This study found considerable differences in pathotype prevalence of leaf rust when compared with the findings of previous studies (Figure 5) (Terefe et al., 2009). However, as pathotype prevalence is mostly a function of where pathotypes were collected, the size of the study and the focus of the study (previous studies had excluded triticale and only sampled rust from wheat cultivars), this is not particularly significant. The inability to definitively assign a specific pathotype to every isolate of leaf rust also had an influence on the prevalence of pathotypes. The incidence of pathotypes did not differ greatly when compared with previous studies, indicating that there have been no pathotype shifts and no introductions of new resistance genes. Only three isolates of yellow rust were found and only one pathotype, 6E16A+ was identified making it impossible to draw any conclusions on changes in yellow rust prevalence.

Conclusion

This study has shown that it is possible to conduct molecular marker based studies of wheat rust pathotype prevalence, incidence and distribution. It is possible to distinguish the pathotypes of leaf and yellow rust using only microsatellite markers. It was possible to use these markers to identify which pathotypes are present in field samples, however the presence of novel alleles in the field isolates precluded the assigning of a pathoype (or pathotypes) to each isolate. More work is required to assign these novel alleles to pathotypes.

AFLP markers cannot be used to distinguish field samples because of the presence of plant DNA. It may be possible to convert AFLP markers that are unique to a pathotype to sequence characterised amplified region

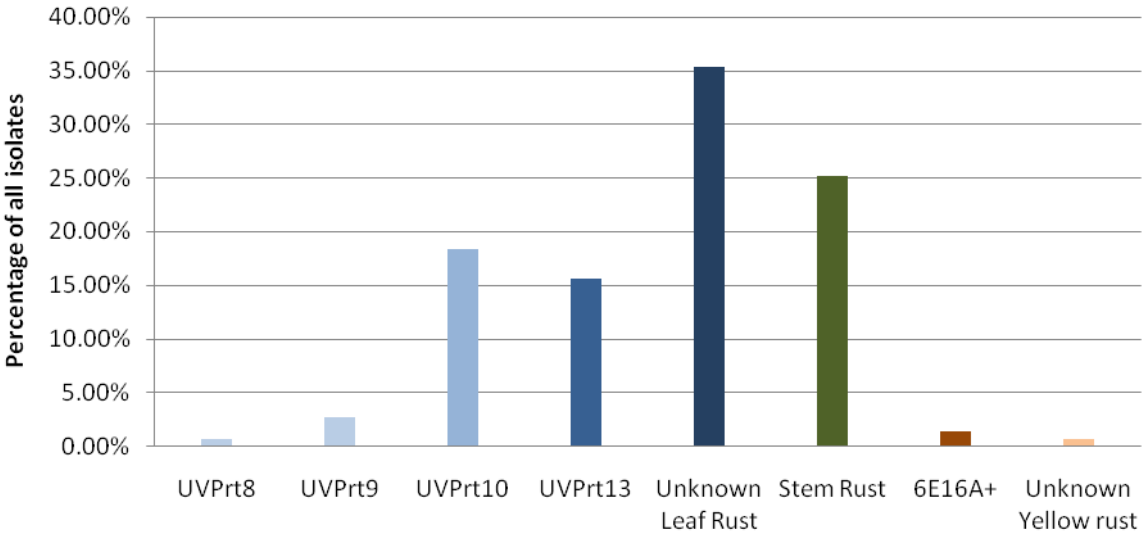

Figure 3. Wheat rust isolates by pathotype.

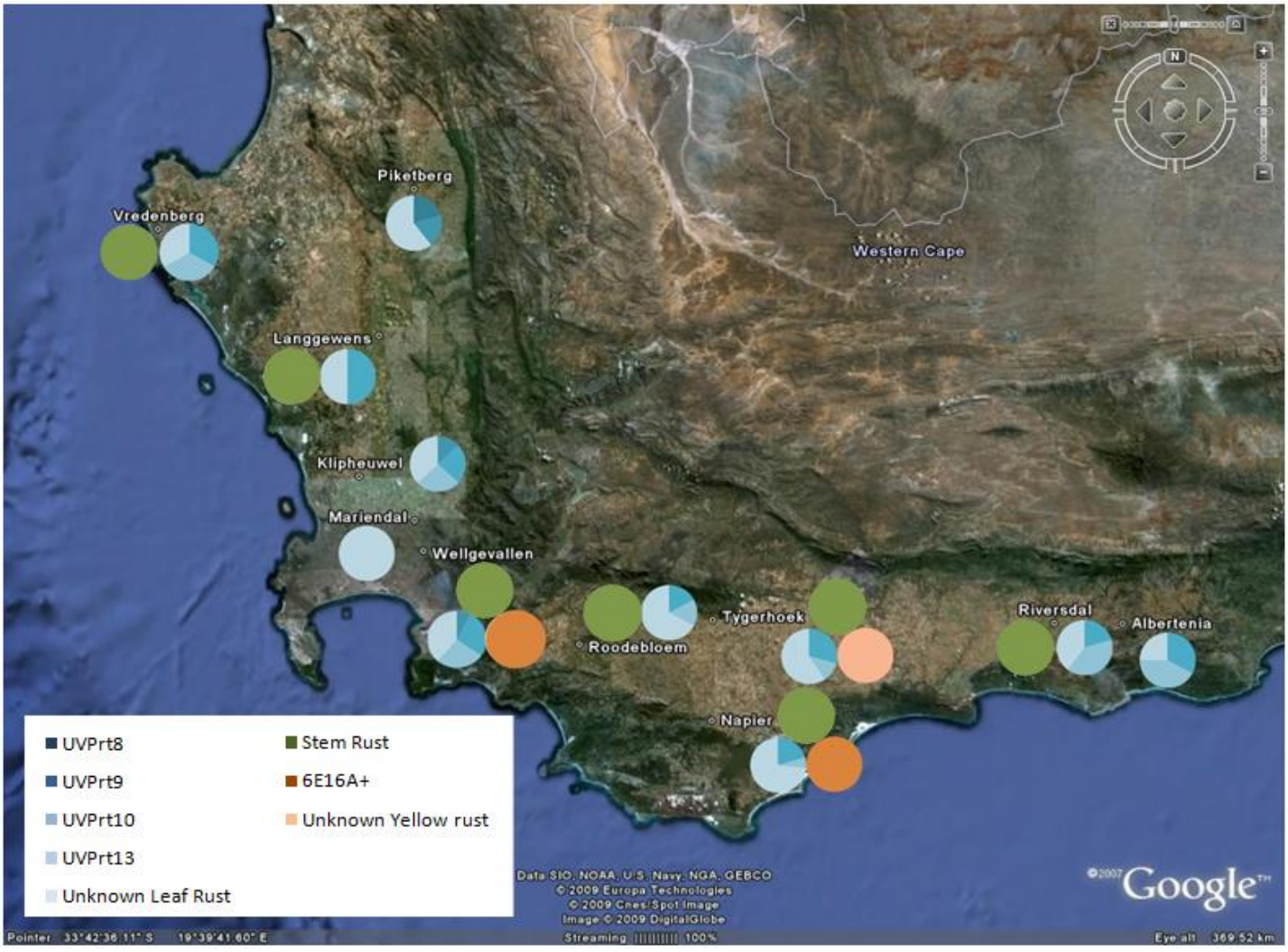

Figure 4. Distribution of wheat rust isolates.

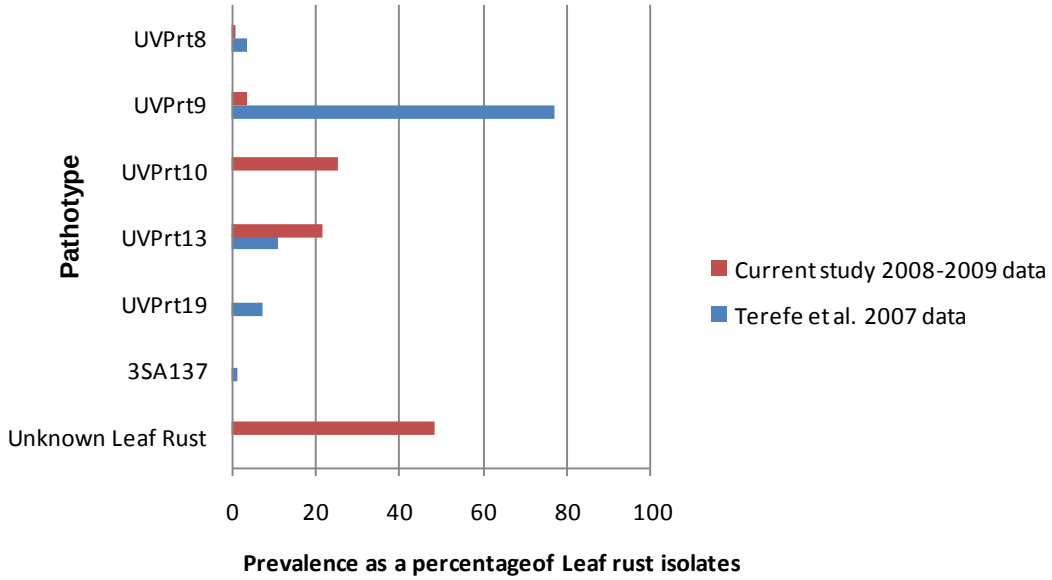

Figure 5. The prevalence of leaf rust isolates found by this study in comparison with the findings of Terefe et al. (2009).

(SCAR) markers. These SCAR markers can then be used to type field isolates. This type of study is not with-out shortcomings, however, multiple infections and novel alleles are confounding factors that will have to be resolved before these techniques can be further developed and used in comprehensive studies of wheat rust diversity and population genetics.

The prevalence of multiple pathotypes with a wide variety of virulence genes in the population shows that breeding for major resistance genes is possibly not the best method as the introduction of cultivars resistant to a single pathotype will simply increase the prevalence of other virulent pathotypes. Rather, breeding programmes should focus on introducing quantitative resistance which provides some resistance to all pathotypes.

REFERENCES

Barnes CW, Szabo LJ (2007). Detection and identification of four common rust pathogens of cereals and grasses using Real-Time polymerase Chain Reaction. Phytopathol., 97, 717-727.

Boshoff WH, Pretorius ZA, Van Niekerk BD (2003). Fungicide efficacy and the impact of stripe rust on spring and winter wheat in South Africa. South Afr. J. Plant. Soil 20: 11-17.

Brown JK (1996). The choice of molecular marker methods for population genetic studies of plant pathogens. N. Phytol. 133: 138-195.

Cavalli-Sforza L, Edwards A (1967). Phylogenetic analysis: models and estimation procedure. Am. J. Human. Genet., 19: 233-257.

Chen RS, Boeger JM, Mcdonald BA (1994). Genetic stability in a population of a plant pathogenic fungus over time. Mol. Ecol. 3: 209-278.

Cuomo C, Sakthikumar S, Grabherr M, Heiman H, Mauceli E, Chen Z, Young S, Zeng Q, Birren B, Bakkeren G, Chen X, Fellers J, Hulbert S, Szabo L (2010). Comparative genomics of wheat rust fungi. BGRI Technical Workshop, St Petersberg, Russia.

Duvellier E, Singh RP, Nicol JM (2007). The challenges of maintaining wheat productivity: pests, diseases and potential epidemics. Euphytica. 157: 417-430.

Enjalbert J, Duan X, Giraud T, Vautrin D, De Vallavielle-Pope C, Solignac M (2002). Isolation of tweleve microsatellite loci, using an enrichment protocol in the phytopathogenic fungus Puccinia striiformis f. sp.tritici. Mol. Ecol. Notes 2: 563-565.

Harvey PR, Langridge P, Marshall DR (2001). Genetic drift and host-mediated selection cause genetic differentiation among Gaeumannomyces graminis populations infecting cereals in Southern Australia. Mycol. Res. 105: 927-937.

Honing J (2007). Evaluation and implementation of DNA-based diagnostic methodology to distinguish wheat genotypes.MSc Thesis.University of Stellenbosch, South Africa.

Keiper FJ, Haque MS, Haydenden MJ, Park RF (2006). Genetic diversity in Australian populations of Puccinia graminis f. sp. avenae.Phytopathol. 96: 96-104.

Keller SM, Mcdermott JM, Pettway RE, Wolfe MS, Mcdonald BA (1997a). Gene flow and sexual reproduction in the wheat glume blotch pathogen Phaeosphaeria nodorum. Phytopathol. 87, 353-358

Keller SM., Wolfe MS, Mcdermott JM, Mcdonald BA (1997b). High genetic similarity among populations of Phaeosphaeria nodorum across wheat cultivars and regions in Switzerland. Phytopathol., 87: 1134-1139.

Kolmer JA, Liu JQ, Sies M (1994). Virulence and molecular polymerphism in Puccinia recondite f. sp. tritici in Canada. Phytopathol., 85, 276-285.

Kolmer JA (2013). Leaf rust of wheat: Pathogen biology, variation and host resistance. Forests, 4: 70-84.

Kolmer JA, Hanzalova A, Goyeau H, Bayles R, Morgourov A (2013). Genetic differentiation of the wheat leaf rust fungus *Puccinia triticina* in Europe. Plant Pathol. 62: 21-31.

Li ZF, Xia XC, He ZH, Li X, Zhang LJ, Wang HY, Meng QF, Yang WX, Li, GI, Liu DQ (2010). Seedling and slow rusting resistance to leaf rust in Chinese wheat cultivars. Plant Dis. 94(1): 45-53

Liu J, Muse S (2005). Power Marker: Integrated analysis environment for genetic marker data. Bioinformat., 21. 2128-2129.

Liu JQ, Kolmer JA (1998). Genetics of stem rust resistance in wheatcus Pasqua and AC Taber. Phytopathol. 96: 96-104.

Liu M, Szabo LJ, Hambleton S, Anikster Y, Kolmer JA (in press). Molecular phylogenetic relationships of the brown leaf rust fungi on wheat, rye and other grasses. Plant Dis.

Mccartney HA, Foster SJ, Fraaije BA, Ward E (2003). Molecular diagnostics for fungal plant pathogens. Pest Manage. Sci. 59: 129-142.

Mcdonald BA, Linde C (2002). The population genetics of plant

pathogens and breeding strategies for durable resistance. Euphytica, 124, 163-180.
Murray TD, Parry DW, Cattlin ND (1998). A colour handbook of diseases of small grain cereal crops. Manson Publications. London.
Park RF, Jahoor A, Felsenstein FG (2000). Population structure of Puccinia recondita in Western Europe during 1995, as assessed by variability in pathogenicity and molecular markers. J. Phytopathol., 148: 169-179.
Pretorius ZA, Pakendorf KW, Marias GF, Prins R, Komen JS (2007). Challenges for sustainable cereal rust control in South Africa. Aus. J. Agric. Res. 58: 593-601.
Schena L, Nigro F, Ippolito A, Gallitelli D (2004). Real-time quantitative PCR: a new technology to detect and study phytopathogenic and antagonistic fungi. Eur. J. Plant. Pathol., 84, 893-908.
Shan W, Chen S, Kang Z, Li Z (1998). Genetic diversity in Puccinia striiformis Westend. f. sp. tritici revealed by pathogen genome-specific repetitive sequence. Canad. J. Bot. 76: 587-595.
Szabo LJ (2007). Development of simple sequence repeat markers for the plant pathogenic rust fungus, *Puccinia graminis*. Mol. Ecol. Notes 7, 92-94.
Szabo LJ, Kolmer JA (2007). Development of simple sequence repeat markers for the plant pathogenic rust fungus, *Puccinia triticina*. Mol. Ecol. Notes 7: 708-710.
Takeszaki N, Nei M (1996). Genetic distances and reconstruction phylogenetic trees from microsatellite DNA. Genet. 144: 389-399.
Terefe T, Paul I, Mebalo J, Naiker K, Meyer L (2009). Occurance and pathogenicity of Puccinia triticina on wheat in South Africa during 2007. South Afr. J. Plant. Soil, 26, 51-53.
Terefe T, Pretorius ZA, Paul I, Mebalo J, Meyer L, Naiker K (2010). Occurrence and pathogenicity of *Puccinia graminis* f. sp. *tritici* on wheat in South Africa during 2007 and 2008. S. Afr. J. Plant Soil, 27(2): 163-167.
Terefe T, Pretorius ZA, Bender CM, Visser B, Herselman L, Negussie TG (2011). First report of a new wheat Leaf Rust (*Puccinia triticina)* race with virulence for Lr12, 13 and 37 in South Africa. Plant Dis., 95(5): 611.
Visser B, Herselman L, Pretorius ZA (2009). Genetic comparison of Ug99 with selected South African races of Puccinia graminis f. sp. tritici. Mol. Plant. Pathol., 10, 213-222.
Zhan J, Mundt CC, Mcdonald BA (1998). Measuring migration and sexual reproduction in field populations of *Mycoshaerella graminicola*. Phytopathol. 88: 1130-1137.
Zhong S, Leng Y, Friesen L, Faris JD, Szabo LJ (2009). Development and characterization of Expressed Sequence Tag-derived micro-satellite markers for the wheat pathogenic Stem rust fungus Puccinia graminis f. sp. tritici. Phytopathol. 99: 282-289.
Zhu Y, Chen H, Fan J, Wang Y, Li Y, Chen J, Fan J, Yang S, Hu L, Leung H, Mew TW, Teng PS, Wang Z, Mundt CC (2000). Genetic diversity and disease control in rice. Nature 406: 718-722.