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ORIGINAL PAPER

Races of *Puccinia triticina* detected on wheat in Zimbabwe, Zambia and Malawi and regional germplasm responses

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Abstract To identify races of *Puccinia triticina* in southern Africa, samples of infected wheat leaves obtained from Zimbabwe, Zambia and Malawi were analysed at the University of the Free State, Bloemfontein and the Agricultural Research Council-Small Grain Institute, Bethlehem, South Africa (SA). Four races were identified from 63 isolates obtained during 2011–2013. Using the North American notation, these races coded to MCDS (74.6 %), TCPS (12.7 %), FBPT (6.3 %) and SCDS (6.3 %). MCDS and TCPS occurred in both Zimbabwe and Zambia whereas FBPT and SCDS were only detected in Zimbabwe and Malawi, respectively. Three of these races (MCDS, FPBT and SCDS) are also known in SA. SSR analysis of races detected in southern Africa suggested that MCDS and FPBT are more closely related to CCPS (3SA45), a race identified in SA in 2009. Occurrence of similar races across southern Africa indicates migration of inoculum between countries, and highlights the need for each country to monitor and share information on virulence changes in the region. In seedling tests, over 72 % of Zimbabwean commercial cultivars were susceptible to race TCPS which occurred in both Zimbabwe and Zambia. To predict occurrence of adult plant resistance (APR) in these cultivars, they were tested for the presence of gene *Lr34* which confers durable resistance to multiple fungal pathogens. Only three cultivars were positive for this gene suggesting that most of the current Zimbabwean commercial cultivars may be susceptible to leaf rust both as seedlings and adult plants, assuming the absence of other APR genes. Three cultivars and 15 breeding lines, all highly resistant as seedlings across races, carried *Lr19*. One line contained *Lr19* and *Lr34*. It is suggested that sources of race non-specific resistance genes be included in wheat breeding programs in Zimbabwe.

Keywords Leaf rust · *Puccinia triticina* · Simple sequence repeats · *Triticum aestivum* · Wheat

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Introduction

Three new races of *Puccinia triticina* Erikss., coded as CCPS, MCDS and FBPT, have been detected in South Africa since 2009 (Terefe et al. 2014a, b). Based on simple sequence repeat (SSR) analysis and comparison with older South African wheat leaf rust races, it was suggested that all three races were most likely foreign introductions (Terefe et al. 2014a, b). One hypothesis is that wind-blown urediniospores reach South African wheat fields from neighbouring countries. In other pathosystems, e.g. wheat stem rust caused by *P. graminis* Pers.

evident, single-pustule isolates were sub-cultured and re-inoculated onto differential lines. At least two differential sets were scored per isolate and where infection types were not clear more replicates were done. Based on the response of differential lines isolates were classified into races using the North American letter code nomenclature system (Huerta-Espino et al. 2011). Where appropriate, ARC South African (3SA_) race codes (Terefe et al. 2014a) were added.

SSR analysis of leaf rust races

SSR markers were used to fingerprint five leaf rust collections, each consisting of a number of single pustule isolates, from Zimbabwe (isolates Zim001-12; Z13-1), Zambia (Zam12-003) and Malawi (Mal1/1; Mal2/1), respectively. For the Zimbabwean and Zambian samples, three to four single-pustule isolates were prepared and DNA was extracted from germinated spores and mycelium using a modified cetyl trimethylammonium bromide (CTAB) protocol (Visser et al. 2009). DNA was extracted from infected leaf tissue for the two Malawian isolates. As controls, genomic DNA from South African races 3SA122, 3SA125, 3SA129, 3SA132, 3SA133, 3SA134, 3SA137, 3SA140, 3SA144, 3SA145 (Visser et al. 2012), 3SA146 (Terefe et al. 2014a) and 3SA147 (Terefe et al. 2014b) was used. SSR analysis was done using 12 previously described SSR markers, namely PtSSR50, PtSSR61, PtSSR76, PtSSR91, PtSSR92 PtSSR152, PtSSR158, PtSSR161, PtSSR164, PtSSR173, PtSSR184 and PtSSR186 (Szabo and Kolmer 2007). PCR amplification, DNA separation, silver staining and data analysis were done as described by Visser et al. (2012).

Structure 2.3.4 (Pritchard et al. 2000) was used to determine the number of leaf rust populations represented by the isolates tested. These results were then used as the basis for AMOVA using Arlequin ver. 3.5 (Excoffier et al. 2005). Structure software was used to estimate the 'true' number of sub-populations (K) without prior knowledge of the population by using the admixture model with inference of alpha from the data together with the allele frequency model, where allele frequencies were correlated. Lambda was set to one. The procedure of Evanno et al. (2005), which determines an ad hoc statistic ΔK based on the rate of change in the log probability of data between successive K values, was followed. The assumed number of populations varied from one to ten. The programme was run with a burn-in period length and Monte Carlo Markov Chain (MCMC) iterations of 10,000 for $K=1$ to $K=10$ and repeated for ten runs per K value. After the calculation of $\Delta K=3$, the analysis was repeated with $K=3$ and both a burn-in period and MCMC replications of 900,000 to verify consistency.

A Mantel test was performed in Arlequin ver. 3.5 to determine the correlation between phenotypic and genotypic data. Only isolates for which the complete four-set North American race codes have been determined were included in the analysis [races 3SA132 (SDDS), 3SA133 (PDRS), 3SA137 (SCDS), 3SA140 (SFDS), 3SA144 (SDDN), 3SA145 (CCPS), 3SA146 (MCDS) and 3SA147 (FBPT), as well as isolates Zim001_12 (FBPT), Z13-1 (MCDS), Mal1/1 (SCDS) and Zam12_003 (TCPS)]. Both the phenotypic and genotypic distance matrices were calculated based on binary data sets and using the Jaccard similarity coefficient. The two distance matrices were then tested for correlation.

Response of Zimbabwean wheat germplasm

To assess the response of 50 Zimbabwean wheat cultivars and lines to the predominant leaf rust races in the region, greenhouse seedling tests were undertaken with 3SA137 (SCDS), 3SA140 (SFDS), 3SA145 (CCPS), 3SA146 (MCDS), 3SA147 (FBPT) and TCPS. Although 3SA140 and 3SA145 were not detected in the present study they are regularly collected in South Africa (Terefe et al. 2014a). Plant growth, inoculation and assessment procedures were the same as for race analyses. All tests were done in duplicate. In addition, the germplasm collection was evaluated in the field for adult plant response to 3SA146 (MCDS) as part of the annual UFS rust nursery at Greytown, South Africa in 2012 and 2013. Race 3SA146 was selected based on its common occurrence in southern Africa. Entries were sown in 1 m row plots spaced 75 cm apart. The nursery was inoculated with 3SA146 by spraying a spore-oil suspension onto spreader rows during the tillering stage.

Lr34 and *Lr19* marker assays

Genomic DNA was extracted from three individual plants of each entry using a sodium dodecyl sulphate extraction protocol as developed by Pallotta et al. (2003) and described in detail in Agenbag (2012). Kariega was included as the *Lr34* positive control (Prins et al. 2011). The co-dominant marker, *cssfr6*, developed from a single nucleotide polymorphism in exon 12 of the gene (Lagudah et al. 2009) was used to predict the presence or absence of *Lr34*. A 10 μl PCR reaction containing 50 ng DNA, 5 pmol FAM-labelled forward primer, 5 pmol unlabelled reverse primer, 0.2 mM dNTPs (KapaBiosystems), 1× GoTaq DNA buffer (Promega Corporation), 1.5 mM $MgCl_2$ and 0.25 U of GoTaq polymerase (Promega Corporation) was performed on a GeneAmp PCR system 9700 (Applied Biosystems). Standard cycling conditions were used with an annealing temperature of 60 °C. PCR products were run on a 3730*xl* Genetic Analyzer (Applied Biosystems) using GeneScan™-1200LIZ® as internal size standard. GeneMapper V4 software (Applied Biosystems) was used to analyse the data.

A subset of lines, displaying a typical fleck infection type, was analysed for the presence of *Lr19*. Amplification of the

gene was achieved with the dominant Lr19STS$_{130}$ marker (Prins et al. 2001) in a CFX96 Touch™ Real-time PCR machine (BIO-RAD). A 10 µl PCR reaction was performed with 1× KAPA™ HRM Fast PCR Kit (KAPA BIOSYSTEMS), 50 ng template DNA, 2.5 mM $MgCl_2$ and 5 pmol of forward and reverse primers. A non-template control was included as well as *+Lr19* and *–Lr19* controls. The following PCR program was used: 3 min at 95 °C, followed by 40 cycles of 5 s at 95 °C, 30 s at 60 °C. Once amplification was completed, a final step of 1 min at 95 °C and 10 min at 40 °C was done before a melt curve analysis was performed by ramping temperature from 70 to 90 °C, rising by 0.2 °C each step of 2 s with continuous acquisition of fluorescence. Automated genotype calling software (CFX Manager™ Software v 3.1, BIO-RAD) was used to determine the genotypes of individual lines according to the melting temperatures.

Results

Race analysis

Four races of *P. triticina* were identified amongst the 63 isolates characterized on differential lines (Table 1). Using the North American notation, these races coded to MCDS (avirulence/virulence formula: *Lr2a, 2c, 3 ka, 9, 11, 16, 18, 24, 30/1, 3a, 10, 14a, 17, 26, B*) (74.6 %), TCPS (avirulence/virulence formula: *Lr9, 11, 16, 18, 24/1, 2a, 2c, 3a, 3 ka, 10, 14a, 17, 26, 30, B*) (12.7 %), FBPT (avirulence/virulence formula: *Lr1, 2a, 9, 11, 16, 24, 26/2c, 3, 3 ka, 10, 14a, 17, 18, 30, B*) (6.3 %) and SCDS (avirulence/virulence formula: *3a, 3 ka, 9, 11, 16, 18, 24, 30/1, 2a, 2c, 10, 14a, 17, 26, B*) (6.3 %). MCDS and TCPS occurred in both Zimbabwe and Zambia whereas FBPT and SCDS were only detected in Zimbabwe and Malawi, respectively. *Lr11* produced an intermediate infection type to TCPS, a response which was inconsistent between different replications in time, especially if scored 14 days after inoculation. The low reaction was confirmed in several experiments, including tests at 18 °C which was the best environment to distinguish between infection types of *Lr11* and the susceptible control Thatcher. Representative isolates of the four races have been lodged in the rust collection of the Department of Plant Sciences, University of the Free State.

SSR analysis of leaf rust races

Following SSR fingerprinting, a dendrogram was constructed in NTSYS-pc version 2.21r using the 33 alleles generated for the 25 representative and control isolates (Fig. 1). A good fit (r=0.927) was found between Jaccard's coefficient matrix and the symmetrical matrix produced from the UPGMA-based dendrogram. Isolates were divided into two major clusters (A and B) sharing 42 % genetic similarity, with cluster A being further divided into two sub-clusters (a and b) sharing 48 % genetic similarity. Sub-cluster b contained the older South African leaf rust races and the two isolates collected from Malawi. The Malawian samples were identical and shared 84 % similarity with five of the older South African races. These five races (3SA132, 3SA134, 3SA137, 3SA140 and 3SA144) were identical. Further attempts to distinguish between these five races using ten additional SSR markers (Wang et al. 2010) were unsuccessful.

Sub-cluster a contained five South African races of which three (3SA145, 3SA146 and 3SA147) are thought to be recent introductions into the country. Two isolates sub-cultured from Z13-1 collected in Zimbabwe shared 88 % similarity with 3SA146, while all four Zim001_12 single pustules were identical to 3SA147. Cluster B contained only one of the original South African races (3SA133) that shared 59 % similarity with all four single pustule isolates of Zam12_003 and the third Z13-1 isolate. The latter isolates were identical. The fourth single-pustule isolate of Z13-1 was excluded from the study, as both the infection type profile and SSR analysis indicated that it was a mixed sample containing spores from both Z13-1.1 and Z13-1.2 (results not shown).

Structure analysis (results not shown) of the isolates suggested the presence of three distinct populations which correlated with sub clusters a, b and B in the dendrogram. When these three populations were used for the AMOVA, 67 % of the variation could be attributed to variation between populations and 33 % to variation within populations. F_{ST} values of 0.61 and higher (P<0.01) indicated genetic differentiation between the three populations. The Mantel test revealed a correlation coefficient of 0.78 (P<0.01) between the phenotypic and genotypic data.

Response of Zimbabwean wheat germplasm

Twenty one entries were resistant to all races in the seedling assay (Table 2). Eighteen of these showed complete resistance (fleck infection types) with W1421/6/6, W1494/6/1, W2500/6/3 and W2234/6/18 being mixed for leaf rust reaction. SC Scan, W2316/6/21 and W5/2008 had intermediate infection types to the races tested. All 18 entries exhibiting fleck reactions across the array of races tested positive for the *Lr19* marker (Table 2). Being a dominant marker the presence of *Lr19* was revealed by a melt peak observed at 84–84.5 °C, whilst the negative and non-template controls showed no amplification or melt curve/peak. Six control lines not expected to contain *Lr19*, viz. SC Nduna, SC Stallion, SC Shine, SC Scan, W2316/6/21 and W5/2008, tested negative for the marker. Race TCPS produced infection types of 3 and higher on most entries (42 %) followed by MCDS (37 %), and SCDS

Table 1 Races of *Puccinia triticina* detected in Zimbabwe, Zambia and Malawi from 2011 to 2013

Year	Country	Race	Locality	No. of isolates	Avirulence / virulence profile
2011	Zimbabwe	MCDS	Marondera, Rusape, Mutare, Nyanga, Kujanga-Nyanyazi irrigation scheme, Chisumbanje, Chiredzi, Masvingo, Goromonzi, Shamva, Harare	31	*Lr2a, 2c, 3ka, 9, 11, 16, 18, 24, 30 / 1, 3a, 10, 14a, 17, 26, B*
	Zimbabwe	TCPS	Goromonzi	4	*Lr9, 11, 16, 18, 24 / 1, 2a, 2c, 3a, 3ka, 10, 14a, 17, 26, 30, B*
2012	Zimbabwe	MCDS	Harare (Gwebi), Shamva, Save Valley, Birchenough, Mutare, Nyanga,	9	As above
	Zimbabwe	FBPT	Harare (Gwebi)	4	*Lr1, 2a, 9, 11, 16, 24, 26 / 2c, 3, 3ka, 10, 14a, 17, 18, 30, B*
	Zambia	MCDS	MRI Farm (Chongwe), Zambeef Farms (Chiawa Estates, Kafue), Syringa Farms (Mazabuka)	7	As above
	Zambia	TCPS	Zamseed Farm (Chisamba)	4	As above
2013	Malawi	SCDS	Tsangano	4	*Lr3a, 3ka, 9, 11, 16, 18, 24, 30 / 1, 2a, 2c, 10, 14a, 17, 26, B*

and SFDS (both 27 %). CCPS (10 %) and FBPT (12 %) were least virulent on the germplasm tested.

Over two years of field evaluation with MCDS, SC Shine (80–100S), SC Scarlet (90S), Sengwa (90S) and Ruya (80–90S) were highly susceptible (Table 2). Flag leaf scores of the remaining entries varied between 0 and 50S. Due to cooler and wetter conditions in 2013 infection levels were lower than the previous year with specifically SC Stallion, Pote and Loerie III showing reduced severities in the second season. The majority of entries with *Lr19* showed an immune phenotype, except lines W2166/6/1 and W2308/6/4 which had a TR in 2012. According to the *cssfr6* marker only cultivars SC Non-Sprout, SC Sahai, SC Scan and breeding line W2486/6/18 tested positive for the adult-plant resistance gene *Lr34*. Leaf rust severity on these entries did not exceed 30 %.

Discussion

Of the four races detected in this study, three (MCDS, FPBT and SCDS) are known in South Africa. Race MCDS (3SA146) was identified in 2010 and has been commonly found since then (Terefe et al. 2014a). This race is virulent for the APR genes *Lr12*, *Lr13* and *Lr37*. Its virulence on *Lr37* distinguishes it from all previously described races in South Africa, except CCPS identified in 2009. Race FPBT (3SA147) which was found in samples collected in Zimbabwe in 2012, was also detected in South Africa in 2010 (Terefe et al. 2014b). Race SCDS was found in Zambia and Zimbabwe in 1986 (Pretorius and Purchase 1990). In South Africa, it was first identified in 1988 and has been frequently recovered since then (Pretorius et al. 1990; Terefe et al. 2009).

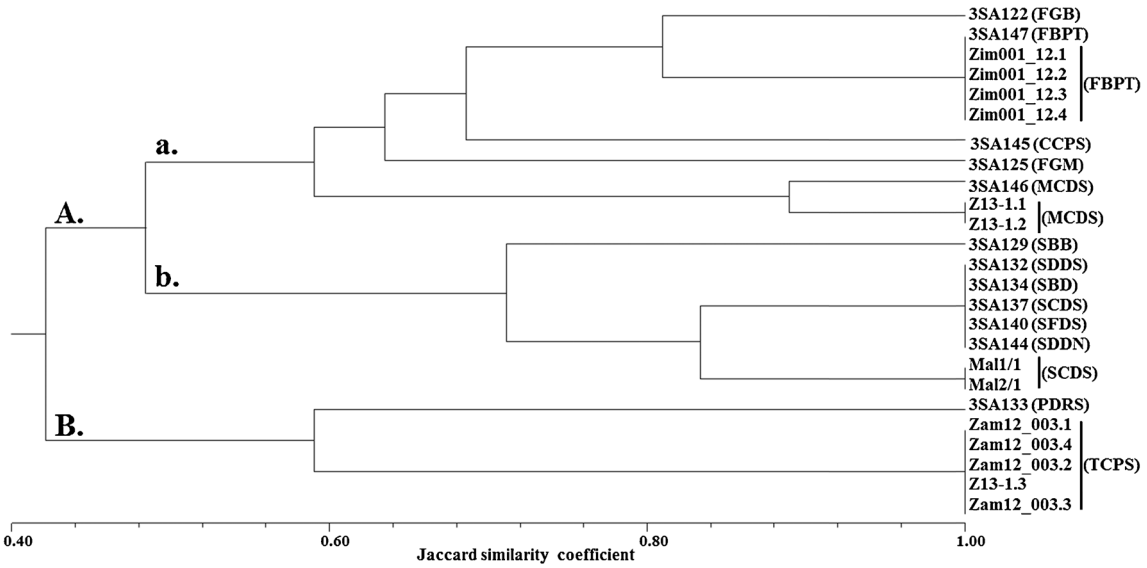

Fig. 1 Genetic comparison of leaf rust isolates collected in Zambia, Zimbabwe and Malawi with selected South African races. The dendrogram was based on 33 alleles generated by 12 SSR markers, using UPGMA clustering and the Jaccard similarity coefficient (Jaccard 1908). North American race notations are indicated in brackets

Table 2 Seedling infection types[a] and adult plant field scores[b] of Zimbabwean wheat cultivars and lines to races of *Puccinia triticina* detected in southern Africa

Entry[c]	Status	Source	Race (North American / ARC-SGI notation)						Field scores (MCDS)	
			SFDS/ 3SA140	CCPS/ 3SA145	MCDS/ 3SA146	FBPT/ 3SA147	SCDS/ 3SA137	TCPS	2012	2013
SC Nduna	Commercial	Seed-Co	2	2+	4	2+	3+	4	40S	20MSS
SC Smart *Lr19*	Commercial	Seed-Co	;	;	;	;	;	;	0	0
SC Stallion	Commercial	Seed-Co	3+	;	4	;	4	4	50S	TS
SC Shield *Lr19*	Commercial	Seed-Co	;	;	;	;	;	;	0	0
SC Shungu	Commercial	Seed-Co	2+	1−	2	1−	3	4	0	10MS
SC Sekuru	Commercial	Seed-Co	;	;	;1−	;1−	;/;1	4	20S	10MS
SC Shorty	Commercial	Seed-Co	;1+	2	3	;1−	;	4	30S	10MS
SC Sky *Lr19*	Commercial	Seed-Co	;	;	;	;	;	;	0	0
SC Shangwa	Commercial	Seed-Co	2+	1	3	;1	4	3	0	0
SC Non-Sprout*Lr34*	Commercial	Seed-Co	;1	3	3+	3	;/4	4	30S	10S
SC Sahai *Lr34*	Commercial	Seed-Co	4	2+	4	2+	4	4	20S	20MS
SC Shine	Commercial	Seed-Co	;1−	3+	3+	1	;	4	100S	80S
SC Scan *Lr34*	Old	Seed-Co	2−	1	1/3	1	2	2	0	TMS
Sengwa	Old	CBI	X	4	4	4	;	4	90S	90S
Ruya	Old	CBI	3	;	4	;	;	4	90S	80S
Nata	Old	CBI	4	;1	2	;	4	4	40S	10MS
Deka	Old	CBI	2	1	1	1	4	4	30S	20MS
W2045/6/13 *Lr19*	Experimental	Seed-Co	;	;	;	;	;	;	0	0
W1284/6/16 *Lr19*	Experimental	Seed-Co	;	;	;	;	;	;	0	0
W1421/6/6 *Lr19*	Experimental	Seed-Co	;	;	;	;	;	;/3	0	0
W1494/6/1 *Lr19*	Experimental	Seed-Co	;	;	;	;	;/4	;	0	0
W1973/6/3 *Lr19*	Experimental	Seed-Co	;	;	;	;	;	;	0	0
W1975/6/9 *Lr19*	Experimental	Seed-Co	;	;	;	;	;	;	No score (YR)	No score (YR)
Kana	Commercial	CBI	2	;	4	;	;	33C	No score (YR)	No score (YR)
Insiza	Commercial	CBI	;1	1+	3+	2−	;	4	No score (YR)	No score (YR)
Kame	Commercial	CBI	;/4	3	3+	3	X+	X	No score (YR)	No score (YR)
Dande	Commercial	CBI	2+	3	3+	4	X+	4	30S	20S
Pan3492	Commercial	Pannar	4	;1−	;1−	;	;1–/3	;n	No score (YR)	No score (YR)
Pote	Old	CBI	–	–	–	–	–	–	50S	10S
SC Scarlet	Old	Seed-Co	4	0	3+	;	4	4	90S	90S
W2444/6/7 *Lr19*	Experimental	Seed-Co	;	;	;	;	;	;	0	0
W2506/6/32	Experimental	Seed-Co	4	;	;1−	;	;	;1	0	NR
W2316/6/21	Experimental	Seed-Co	2	;	;1−	;	;	;	0	0
W2486/6/18 *Lr19 Lr34*	Experimental	Seed-Co	;	;	;	;	;	;	0	0
W2445/6/12	Experimental	Seed-Co	3	;	;1	;	;1	;	0	0
W5/2008	Experimental	Seed-Co	1	;	;1/2	;1−	;1	;1	0	5MR
W13/2002	Experimental	Seed-Co	;1	4	4	;/3	;1	3+	10S	0
W46/2003	Experimental	Seed-Co	1	2	4	;/3	;1	3	5S	0
W2444/6/8 *Lr19*	Experimental	Seed-Co	;	;	;	;	;	;	0	0
W2397/6/8 *Lr19*	Experimental	Seed-Co	;	;	;	;	;	;	0	0
W2500/6/3 *Lr19*	Experimental	Seed-Co	;	;	;	;	;/4	;	No score (YR)	No score (YR)
Loerie III	Commercial	Zambia	2	;1	4	;	4	4	50S	10S
W2469/6/3	Experimental	Seed-Co	4	;	;1−	;	;1	;	No score (YR)	No score (YR)
W2488/6/3	Experimental	Seed-Co	3	1−	1	;	;	;1	0	0
W2500/6/40 *Lr19*	Experimental	Seed-Co	;	;	;	;	;	;	No score (YR)	No score (YR)

Table 2 (continued)

Entry[c]	Status	Source	Race (North American / ARC-SGI notation)						Field scores (MCDS)	
			SFDS/ 3SA140	CCPS/ 3SA145	MCDS/ 3SA146	FBPT/ 3SA147	SCDS/ 3SA137	TCPS	2012	2013
W2234/6/18 *Lr19*	Experimental	Seed-Co	;	;	;/2	;	;	;	0	0
W2166/6/1 *Lr19*	Experimental	Seed-Co	;	;	;	;	;	;	TR	0
W2316/6/17	Experimental	Seed-Co	4	;	;1−	;	;1	;	5S	0
W2320/6/24	Experimental	Seed-Co	4	;1−	1	;	;1	;	0	0
W2308/6/4 *Lr19*	Experimental	Seed-Co	;	;	;	;	;	;	TR	0
Line 37–07	Field check								80S–100S	80S–100S

[a] Seedling infection types were scored according to a 0 to 4 scale (McIntosh et al. 1995). Infection types separated by / indicate an entry which is mixed for leaf rust response

[b] Field severity scores qualified by R, MR, MS or S indicate resistance, moderate resistance, moderate susceptibility and susceptibility. YR refers to extensive yellow rust damage preventing a reliable leaf rust score

[c] Entries carrying *Lr19* or *Lr34* according to the $Lr19STS_{130}$ and *cssfr6* markers, respectively, are indicated by superscripts after the cultivar or line name

Virulence and genetic studies suggested that MCDS and FPBT are more closely related to the South African race CCPS (3SA145). This indicates that these three races may have developed from a common ancestor. In addition, occurrence of similar races in different countries of southern Africa suggests that races migrate between countries. The common isolation (75 %) of race MCDS in 2011 and 2012 in the current study, and its recent presence in South Africa, is another example of rust migration on a global scale. According to Kolmer et al. (2013) MCDS was first found in North America in 1996 followed by South America in 1998. This race has now been reported from France and Turkey (Kolmer et al. 2013) and Ethiopia (www.slideshare.net/bgri/2014-bgri-kolmer accessed 8 July 2014).

Results of the present study combined with previous findings imply that the southern African countries including Malawi, Zambia, Zimbabwe, Mozambique and South Africa can be considered one epidemiological zone (Pretorius and Purchase 1990; Mukoyi et al. 2011; Pretorius et al. 2012; Terefe et al. 2014a). At least three clearly differentiated leaf rust populations are present with strong evidence of migration within the region.

This highlights the need for each country to constantly monitor changes in the virulence of rust pathogens and share information at regional level. Information of this nature can assist countries to pro-actively respond by preparing short-term intervention strategies and help in identifying effective resistance sources for variety development. For instance, the virulent race TCPS found in Zambia and Zimbabwe has not yet been detected in Malawi or South Africa. The availability of TCPS allows breeders and pathologists of the two latter countries to determine susceptibility risks before the race arrives.

Over 77 % of Zimbabwean commercial cultivars were susceptible as seedlings to one or more races tested in this study. Most of these cultivars (72 %) were susceptible to race TCPS. To predict occurrence of APR in these cultivars, they were tested for the presence of gene *Lr34* which confers resistance to multiple fungal pathogens. However, only three cultivars (SC Non-Sprout, SC Sahai and SC Scan) were positive for this gene suggesting that some Zimbabwean commercial cultivars could be vulnerable to leaf rust. Unlike cultivars, the majority of the breeding lines were resistant as seedlings to all races. One breeding line, W2486/6/18, carried the durable resistance gene *Lr34*. From the infection types it appears that several lines obtained their resistance from major genes which may be vulnerable to breakdown with the emergence of new races. The highly resistant fleck phenotype observed in many lines resembled *Lr19,* a postulation which was subsequently confirmed by positive amplification of the linked $Lr19STS_{130}$ marker in all these entries. The origin of *Lr19* can be traced to CIMMYT germplasm as all lines containing the gene were obtained from either crosses between CIMMYT lines or between local and CIMMYT lines. Lines W2045/6/13 and W1284/6/16, both possessing *Lr19* (Table 2), were released as SC Serena and SC Select in Zimbabwe in 2012. Although virulence for *Lr19* is generally rare, it has been reported from South America, India and China (Huerta-Espino et al. 2011). The collection should thus be genotyped for the presence of race non-specific genes such as *Lr46* and *Lr67* (Lagudah 2011) to obtain a better understanding of the occurrence of more durable resistance. The use of at least two effective race specific genes in combination with race-nonspecific adult plant resistance genes should help in increasing the durability of leaf rust resistance in future cultivars.

Despite the importance of leaf rust in Zimbabwe and with the exception of South Africa, information on diversity in *P. triticina* in other southern African countries is limited. Pretorius and Purchase (1990) detected six *P. triticina* races

from samples collected in Zimbabwe, Zambia and Malawi in the 1980s. The general lack of wheat leaf rust data is compounded by the shortage of genotypic information on *Lr* genes deployed in Zimbabwean wheat varieties. Variety selection is based on visual assessments of the phenotypic response of lines to natural leaf rust inoculum.

In conclusion, this study has shown similarities in leaf rust races in southern African countries. It is thus feasible to assume that migration is an important driver of rust diversity in the region. The detection of Zimbabwean and Zambian races similar to those recently discovered in South Africa suggests a southerly, step-wise migration pattern of urediniospores in Africa. Coordinated surveys will help to better understand virulence of *P. triticina* on a regional scale and provide a scientific basis for selecting sources of resistance in breeding programs.

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