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Variation in *Puccinia graminis* f. sp. *tritici* detected on wheat and triticale in South Africa from 2009 to 2013

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ABSTRACT

To determine phenotypic diversity of *Puccinia graminis* f. sp. *tritici (Pgt)*, the cause of stem rust of wheat, samples of infected stems were collected during 2009–2013 from commercial wheat fields, experimental plots, and rust trap nurseries across major wheat growing regions of South Africa (SA). *Pgt* races were identified based on their avirulence/virulence profiles on seedlings of 20 standard and five supplemental differential lines. Nine *Pgt* races were identified from 521 isolates pathotyped. Predominant races were TTKSF (2SA88, South African race notation) with 39%–85% frequency and BPGSC + *Sr27,Kiewiet,Satu* (2SA105) with 10%–20% frequency. Race TTKSF is virulent on major resistance genes such as *Sr5*, *Sr6*, *Sr9e*, and *Sr38* and is one of the variants of the highly virulent Ug99 race group originally detected in East Africa. Race TTKSP (2SA106), also a member of the Ug99 lineage, was detected in 2009 and 2010. A new race virulent on *Sr31*, PTKST (2SA107), was detected in 2009. Two new races, TTKSF + *Sr9h* (2SA88 + *Sr9h*) and BFBSC (2SA108), were identified in 2010. Race TTKSF + *Sr9h* is similar to TTKSF except for its virulence on *Sr9h*. Race BFBSC appears related to *Pgt* races characterized by avirulence for *Sr5* and often attacking triticale. Simple sequence repeat (SSR) analysis indicated that race BFBSC forms part of the non-Ug99 group of South African *Pgt* races. Despite some similarity in avirulence/virulence phenotype with the non-Ug99 races, BFBSC represents a third distinct genetic lineage within this group. Genes *Sr13*, *14*, *22*, *25*, *26*, *29*, *32*, *33*, *35*, *36*, *37*, *39*, *42*, and *43* that are effective against the new and other *Pgt* races can be used in resistance breeding in SA. Races like PTKST and TTKSF + *Sr9h* were also reported in other Southern African countries suggesting that they probably spread to SA from neighbouring regions. The new races are additions to nearly 30 *Pgt* races identified since the early 1980s, and suggest continued variability of the *Pgt* population in SA. Therefore, surveys should be conducted regularly to timely detect and manage new races, and utilize the latter in screening and identification of effective sources of resistance.

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1. Introduction

Stem rust caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*) is a major disease of bread wheat (*Triticum aestivum* L.) worldwide. *Pgt* infects wheat stems and leaves and occasionally heads and awns and has the potential to cause significant yield loss in susceptible cultivars. For example, a stem rust epidemic that occurred in the United States of America prior to 1954 caused an average yield loss of over 30% in some regions, and significant losses due to this disease have been recorded in many countries (Dean et al., 2012; Singh et al., 2015). Stem rust has been regularly found in South Africa (SA), sometimes reaching epidemic levels in the winter rainfall wheat growing regions of the Western Cape (Le Roux, 1989; Le Roux and Rijkenberg, 1987), and causing yield losses of over 35% (Pretorius et al., 2007).

Growing resistant cultivars is the most cost effective means of controlling stem rust. However, resistance conferred by race-specific genes is often overcome by the continual emergence of new *Pgt* races. The appearance, adaptation, and spread of the highly virulent stem rust race Ug99 (TTKSK, North American notation) emphasized the importance of *Pgt* variability in global wheat production. Prior to 1999, resistance gene *Sr31* was effective and widely used in breeding in many countries for more than 30 years.

Abbreviations: ARC-SGI, Agricultural Research Council-Small Grain Institute; Pgt, *Puccinia graminis* f. sp. *tritici*; SA, South Africa.

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However, *Sr31* became ineffective against Ug99 which was detected in Uganda in 1998 and described in 1999 (Pretorius et al., 2000). During the past 15 years, not only has Ug99 spread to other countries, it has also evolved to defeat additional genes including *Sr24* by race TTKST (Jin et al., 2008), *Sr36* by TTTSK (Jin et al., 2009) and *SrTmp* by TTKTT and TTKTK (Patpour et al., 2015). A variant of Ug99 with virulence for *Sr9h* has been detected (Pretorius et al., 2012) but combined virulence for *Sr9h* and *Sr31* is not known. So far, one or more of the 11 races in the Ug99 linage have been confirmed in Egypt, Eritrea, Ethiopia, Iran, Kenya, Mozambique, Rwanda, South Africa, Sudan, Tanzania, Uganda, Yemen, and Zimbabwe (Boshoff et al., 2002; Jin et al., 2008, 2009; Mukoyi et al., 2011; Nazari et al., 2009; Pretorius et al., 2000, 2010, 2012; Terefe et al., 2010; Wolday et al., 2011).

Rust monitoring helps to identify new races, which can subsequently be used in risk assessment and resistance breeding. Thus, surveillance has been a major component of the rust resistance breeding programme of the Agricultural Research Council-Small Grain Institute (ARC-SGI) and other breeding companies over the past 35 years. Through surveys conducted since the early 1980s, nearly 30 *Pgt* races were discovered in SA (Boshoff et al., 2000, 2002; Le Roux, 1989; Le Roux and Rijkenberg, 1987; Pretorius et al., 2007; Smith and Le Roux, 1992; Terefe et al., 2010). Each of these races was used to screen sources of resistance to identify appropriate breeding lines, leading to a sustainable availability of resistant germplasm. Many *Pgt* races detected in SA have developed due to genetic mutations in local rust populations (Pretorius et al., 2007). However, some of them were most probably foreign introductions (Pretorius et al., 2010; Visser et al., 2009). *Pgt* will continue to evolve and form new races from time to time, and such new races have the potential to spread between different countries. Therefore, rust monitoring remains an effective strategy in meeting challenges related to virulence changes and in the sustainable management of stem rust through resistance breeding. The main objective of the present study was to determine the phenotypic and genotypic variation among *Pgt* races detected in South Africa from 2009 to 2013.

2. Materials and methods

2.1. Stem rust surveys

Stem rust surveys were conducted during 2009–2013 in commercial wheat fields, experimental plots of breeders, and rust trap nurseries planted across the major wheat growing regions including Free State, KwaZulu-Natal, Northern Cape, Eastern Cape, Western Cape, and Mpumalanga provinces. The number of localities where trap entries were planted during the five seasons varied between 21 in 2011 to 30 in 2009. Trap nurseries included commercial wheat cultivars and several near-isogenic lines containing different stem rust resistance genes. Wheat cultivar McNair 701 was planted at regular intervals amongst trap entries to serve as a universally susceptible host. During all five seasons, the trap nurseries were monitored at least twice between August and December for stem rust signs.

2.2. Race identification

Stem rust samples collected from wheat and triticale (x*Triticosecale*) were processed at ARC-SGI and where appropriate the University of the Free State (UFS). Urediniospores were collected from each sample using a cyclone spore collector. Race analysis was performed in the glasshouse using the modified North American (NA) differential set (Jin et al., 2008). The revised NA set consisted of 20 tester lines: ISr5-Ra (*Sr5*), Cns_T_mono_deriv (*Sr21*), Vernstein (*Sr9e*), ISr7b-Ra (*Sr7b*), ISr11-Ra (*Sr11*), ISr6-Ra (*Sr6*), ISr8a-Ra (*Sr8a*), Acme (*Sr9g*), W2691SrTt-1 (*Sr36*), W2691Sr9b (*Sr9b*), Festiquay (*Sr30*), Renown (*Sr17*), ISr9a-Ra (*Sr9a*), ISr9d-Ra (*Sr9d*), W2691Sr10 (*Sr10*), CnsSrTmp (*SrTmp*), LcSr24Ag (*Sr24*), Sr31/6*LMPG (*Sr31*), VPM 1 or Trident (*Sr38*), and McNair 701 (*SrMcN*). Four supplemental lines, namely Barleta Benvenuto (*Sr8b*), Coorong triticale (*Sr27*), Kiewiet triticale (*SrKiewiet*), and Satu triticale (*SrSatu*), were added as they are useful to differentiate some races commonly found in SA (Pretorius et al., 2007; Terefe et al., 2010). South African wheat cultivar Matlabas was included as a tester for *Sr9h* starting from 2012 (Pretorius et al., 2012). Five to 10 seeds of each differential line were planted in clumps in 10-cm diameter plastic pots (4 entries per pot) filled with steam-sterilized soil. The soil was treated with 10 g L^{-1} water soluble fertilizer containing nitrogen (15%), phosphorous (4.5%) and potassium (26.3%). Prior to inoculation the seedlings were grown under lights in a rust-free environment at 22–25 °C.

Urediniospores collected from each sample were directly inoculated onto differential lines. When mixed races were detected, single-pustule isolates were established and increased before re-inoculation onto differential lines. Seedlings of the differential lines were inoculated with a suspension of urediniospores prepared in mineral oil (Soltrol-170®) seven days after planting (first leaf fully extended). Inoculated seedlings were first dried-off for 2 h and then incubated in a dew chamber at ± 20 °C and 100% relative humidity. Seedlings removed from the dew chamber after incubation for 16–18 h were dried-off for 2 h under light emitted by white fluorescent tubes and then placed in glasshouse cubicles at ± 20 °C. The infection types of the seedlings were assessed using a 0–4 scale 10–14 days after inoculation (Roelfs et al., 1992). Infection types 0–2 were considered low (*Pgt* avirulence) and infection types 3–4 were considered to be high (*Pgt* virulence). Races were identified based on their avirulence/virulence profiles on the differential lines, and named according to North American race nomenclature (Roelfs and Martens, 1988), and SA race notation (Le Roux and Rijkenberg, 1987). The identity of each race was confirmed by repeating all phenotyping tests.

2.3. Genetic analysis

The genetic relationship between race BFBSC and ten South African *Pgt* races, as well as TTKSK (Ug99), was determined using 22 polymorphic simple sequence repeat (SSR) markers (Karaoglu et al., 2013). Urediniospores of three single pustule isolates for BFBSC were germinated for 16 h at 20 °C on sterile dialysis tubing placed on 0.6% (w/v) water agar. Genomic DNA was extracted from the germinated tissue using cetyl trimethylammonium bromide (CTAB; Saghai-Maroof et al., 1984) as previously described (Visser et al., 2009).

Each 10 μl PCR reaction contained 2 ng genomic DNA, 1 μM of each primer, and a 1× concentration of KAPA Taq Ready Mix (KAPA Biosystems, Cape Town, South Africa). The amplification regime was one cycle of 4 min at 94 °C, followed by 35 cycles of 94 °C for 30 s, recommended annealing temperature for 30 s and 72 °C for 30 sec. A 5 min step at 72 °C was finally added. After confirming successful amplification on an 1.2% (w/v) agarose gel, the amplified DNA was separated on a 5% (w/v) denaturing polyacrylamide gel using 1× TBE as running buffer (Sambrook et al., 1989). DNA fragments were visualized using silver staining (Silver Sequence™ DNA Sequencing System; Promega).

A multi-locus allelic data matrix based on SSR allele sizes was generated for all genotypes. STRUCTURE 2.23 software was used to determine the genetic structure of the South African *Pgt* population based on the twelve described races including TTKSK, according to Bayesian model-based clustering without prior knowledge of

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