



Accomplishments in wheat rust research in South Africa

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Rust diseases, although seasonal, have been severe constraints in wheat production in South Africa for almost 300 years. Rust research gained momentum with the institution of annual surveys in the 1980s, followed by race identification, an understanding of rust epidemiology, and eventually a focused collaboration amongst pathologists, breeders and geneticists. Diversity in South African populations of *Puccinia triticina*, *P. graminis* f. sp. *tritici* and *P. striiformis* f. sp. *tritici* has been described and isolates are available to accurately phenotype wheat germplasm and study pathogen populations at national, regional and global levels. Sources of resistance have been, and still are, methodically analysed and molecular marker systems were developed to incorporate, stack and verify complex resistance gene combinations in breeding lines and cultivars. Vigilance, capacity, new technologies, collaboration and sustained funding are critical for maintaining and improving the current research impetus for future management of these important diseases.

Significance:

- Rust diseases threaten wheat crops worldwide, including in South Africa.
- Management of rusts includes regular surveillance, pathogen diversity studies, rigorous screening of wheat germplasm, and efficient breeding and selection for resistance.
- Collaboration among plant pathologists, geneticists and breeders has provided momentum in rust research and control in South Africa in recent years.

Background

The sowing of small grain cereals in South Africa occurred within 2 months after the United (Dutch) East India Company set foot on land in present-day Cape Town in 1652.^{1,2} Crop failures, in particular due to damage caused by heavy rains, wind storms and unadapted cultivars, were common occurrences. Nonetheless, efforts to successfully grow wheat continued and systematically included new production areas, different sowing times, new cultivars – not only from Europe but also from India, and exports when grain supplies allowed. Varietal assessments during the early years provided evidence for the first selection of higher-yielding types in South Africa.² The pioneering wheat cultivars are not well documented, but reference is made of 'white' wheat in 1659, 'Sarut' from India in 1673, 'Roode' and 'Grijs Winter' in 1677, until names based on phenotype (e.g. 'Bloukoring', 'Kleinkoring', 'Baardkoring', 'Zwartbaard', 'Vroeëbaard'), origin (e.g. 'Ciciliaans', 'Bengaalsch'), or growers (e.g. 'Du Toits', 'Niewoudts', 'Tautes') became customary.²

No mention is made of rust during the foundational years of cereal production in South Africa but, according to Theal³, a critical shortage of wheat in 1727 was ascribed in the previous year to rust – a disease known in South Africa only on rye at the time. The regular occurrence of rust led Neethling² to conclude: 'There is no doubt that rust, owing to the severe damage caused, is the most important factor which caused the extinction and origin of varieties in South Africa'. Nhemachena and Kirsten⁴ gave a detailed account of wheat cultivar development in South Africa, Smit et al.⁵ summarised wheat research between 1983 and 2008, and overviews of wheat rust research in South Africa were provided by De Jager⁶, Lombard⁷ and Pretorius et al.⁸ Early milestones were interspecies crosses to transfer stem rust resistance genes to bread wheat (*Triticum aestivum* L.) in 1912 followed by pathotyping isolates of *Puccinia graminis* f. sp. *tritici* Erikss. & E. Henn. (*Pgt*) and *P. triticina* Erikss. (*Pt*) in the 1920s and 1930s.⁸ The establishment of a centre for dedicated small grains research at Bethlehem in 1976, currently named Agricultural Research Council – Small Grain (ARC-SG), resulted in appropriate training in rust methodologies, surveillance, race analysis and germplasm evaluation. These initiatives were expanded with the formation of a rust laboratory at the University of the Free State in 1989.

In recent decades, notable events and initiatives in South African wheat rust research include annual surveys⁸, *Sr24* virulence⁹, the appearance of stripe rust (caused by *P. striiformis* Westend. f. sp. *tritici*, *Pst*)¹⁰, Ug99 stem rust studies¹¹⁻¹⁵, the mapping of quantitative resistance loci^{16,17}, genetic characterisation of *Puccinia* isolates¹⁸⁻²⁰, comprehensive phenotyping of wheat germplasm, and establishment of a marker service laboratory with a particular focus on rust resistance genes (<https://www.cengen.co.za>).

The objective of this review is to provide a summary of recent accomplishments in wheat rust research in South Africa.

Rust surveillance and phenotypic analysis

Surveillance and race typing are routinely conducted by the ARC-SG to determine rust distribution, impact and pathogenicity in the major wheat-producing areas of South Africa. Recent reports of similarities in races between southern African countries have also emphasised the importance of regional sampling.²¹⁻²³

Handling obligate rust fungi in controlled experiments such as race typing or host plant screening requires specific infrastructure. In addition to facilities for plant growth, inoculation and incubation, equipment for collection

and application of small amounts of urediniospores is essential. Because these specialised items are not commercially available, Pretorius et al.²⁴ developed an additive manufacturing process to assemble spore collectors and atomisers through 3D printing. Using these devices, traditional race analysis is done by infecting seedlings of a predetermined (differential) set of wheat host lines with a rust isolate. An appropriate experimental set-up and experience in achieving accurate seedling infection types are essential for reliable phenotyping. Examples of infection types are shown in Figure 1.



Figure 1: Seedling (top, left to right: wheat leaf rust, stem rust and stripe rust) and adult plant phenotypes commonly encountered for leaf rust (second from top), stem rust (third from top) and stripe rust (bottom).

Based on the pathogenicity of an isolate on entries in the differential set, a race (pathotype) name is allocated. Apart from an alpha-numerical code to name leaf and stem rust races in South Africa⁸, the North American system of nomenclature^{25,26} is used to place races in an international context. The standard South African differential set for determining seedling infection types to *Pt* isolates contains 20 entries.²⁷ Except for

Thew (*Lr20*) and Agent (*Lr24*), all *Lr* genes occur in a Thatcher wheat background. New races are further characterised on an additional set containing 23 *Lr* genes.²⁷ Infection types on the lines RL6011 (*Lr12*), CT263 (*Lr13*), RL6044 (*Lr22a*), RL6058 (*Lr34*), RL6082 (*Lr35*) and Thatcher control (*Lr22b*) are determined on flag leaves of adult plants.

No new *Pt* races were detected between 1988 and 2008 in South Africa.^{8,28} This situation changed with the report of race 3SA145 (CCPS North American race code) in 2009, followed by races 3SA146 (MCDS, 2010), 3SA147 (FBPT, 2010), 3SA115 (CBPS, 2012), 3SA10 (CFPS, 2016), 3SA38 (CDPS, 2016) and 3SA248 (CFPS, 2016).²⁷⁻³⁰ The frequency of *Pt* races with virulence to *Lr3*, *Lr12*, *Lr13*, *Lr15*, *Lr26* and *Lr37* is high and varied between 79% and 98% during recent surveys.²⁷ The *Pt* population was dominated for many years by race 3SA133 (PDRS) which initially was common on winter wheat in the Free State. This changed significantly with the appearance of races 3SA145, 3SA146 and 3SA115 which accounted for >80% of isolates typed during the 2012–2016 surveys.²⁷ The more recently described races 3SA38 and 3SA10 are increasing in prevalence and comprised more than 50% of the isolates typed from the 2018 growing season.³¹ *Pt* race MCDS was common in Zimbabwe and Zambia with FBPT and SCDS detected in Zimbabwe and Malawi.²³

Twenty differential wheat lines are used for stem rust pathotyping. Although the resistance genes are similar to the proposal of Jin et al.²⁶, Acme (*Sr9g*), Renown (*Sr17*) and Trident (*Sr38*) have replaced CnSr9g, Combination VII and VPM1, respectively. Additional differentials include Barleta Benvenuto (*Sr8b*), the triticales Coorong (*Sr27*), Kiewiet (*SrKw*) and Satu (*SrSatu*), and either LcSrWst-2Wst (*Sr9h*) or Matlabas (*Sr9h*).³² New races are further characterised on an extended set of tester lines.³³ Although differential lines grown in the field can provide an indication of prevailing *Pgt* races, Boshoff et al.³⁴ showed that certain resistance genes are not well expressed in adult plants whereas other lines contain resistance in addition to that observed in seedling assays.

The most significant change in the *Pgt* population since 2005 was the regular appearance of new races in the Ug99 lineage. African race Ug99, named after the country of first detection (Uganda) and year of description (1999)¹¹, was the first race with virulence for the widely used *Sr31* resistance gene. Its broad virulence and subsequent specialisation in 13 pathotypes have raised serious concerns about sustained wheat production in many regions of the world.¹⁵ Stem rust race 2SA106 (TTKSP North American race code) detected in 2007, 2SA107 (PTKST, 2009), 2SA88+ (TTKSF+, 2010) and 2SA42 (PTKSK, 2017) all show phenotypic similarities to race 2SA88 (TTKSF, 2000), which was the first stem rust race in the Ug99 lineage detected in South Africa.^{12,13,18,21,32,35} These races are phenotypically characterised by differences in virulence for *Sr9h*, *Sr21*, *Sr24* and *Sr31*.^{13,32} *Sr24* and *Sr31* have been reported to occur in South African wheat germplasm³⁶ and virulence was not unexpected. Likewise, the virulence adaptation of TTKSF+ was recently confirmed by the endorsement of *Sr9h* in the wheat cultivar Matlabas.³⁷ Despite being less virulent compared to the more recently detected Ug99 races, TTKSF remains the dominant variant.^{8,31,32,38} Stem rust races TTKSF (2009), TTKSF+ (2010) and PTKST (2010) were also identified in samples collected in Zimbabwe and PTKST was confirmed in Mozambique.^{15,21}

Seedling infection types produced on the World and European differential sets^{39,40}, followed by an A+ or A- suffix to describe virulence or avirulence for the *YrA* gene in Avocet R⁴¹, are used for *Pst* race designations in South Africa. Near-isogenic lines with Avocet S as the recurrent parent are used as additional tester lines for race characterisation and in field plots.⁴² Following the detection of *Pst* race 6E16A- in 1996¹⁰, proposed to be a foreign introduction from Central or Western Asia either by wind or human intervention^{43,44}, there is strong evidence that adaptation to the host genes *Yr25* (race 6E22A-, cultivar Hugenoot, 1998) and *YrA* (6E22A+, PAN 3195, 2005) resulted from selection pressure^{10,19}. The *Pst* population has remained relatively stable since the detection of race 6E22A+ on winter wheat in the eastern Free State in 2005¹⁹ with 6E22A+ persisting as the most dominant race, comprising 58% of isolates in 2018³¹.

The outbreak of stripe rust on irrigated spring wheat in 2018 represented the first report of the disease in Zimbabwe.⁴⁵ Showing virulence to *Yr3a*, *Yr4a*, *Yr9* and *Yr27*, race 30E142A+ was distinctly more virulent on South African wheat cultivars than 6E22A+ and poses a potential threat to the local industry. However, it is not yet known if *Pst* will successfully establish in Zimbabwe and, as anticipated, migrate to South Africa.

It has been suggested that wheat cultivated at a higher elevation in Lesotho during summer serves as a source of *Pst* inoculum for winter-grown crops in South Africa.⁸ Although not customary, some hectares may also be sown to wheat in the Free State during summer. The impact of these formal off-season productions on the epidemiology of the rusts has, however, not been studied in detail. Volunteer wheat has generally been assumed to provide a green bridge for the survival of these biotrophic pathogens between seasons.

Genetic analysis of *Puccinia* isolates

Analysis of *Pt*, *Pgt* and *Pst* with microsatellite markers has contributed to explaining genetic diversity within the three populations. In the absence of functional alternate hosts for wheat rust pathogens in South Africa, wind dispersal, human activities, mutation and possibly somatic recombination are considered as drivers of variation. The South African *Pgt* population consists of two highly diverse genetic lineages.¹⁸ In the absence of viable historical samples in South Africa, the close genetic similarity of members of the non-Ug99 genetic lineage with Australian standard races 21-0 collected in 1954, and 326 and 194 collected in 1969, respectively, suggested that this lineage represents the original South African population.⁴⁶ Included in this lineage are races that are specific for both wheat and triticale. The acquisition of virulence within this group appears to be the result of step-wise mutations.^{32,33} On a global scale, this lineage grouped closest with *Pgt* samples from Pakistan, Czech Republic^{47,48} and Australia due to the proposed movement of urediniospores on high-altitude westerly winds⁴⁶.

The Ug99 lineage on the other hand, first detected in South Africa in 2000 with the description of TTKSF¹², has expanded into five variants^{13,14,21,35}. In contrast to the non-Ug99 lineage, all five South African variants and the original TTKSK¹¹ shared more than 85% genetic similarity and fall within the bigger Ug99 race group from east Africa¹⁵. In a recent study, Li et al.⁴⁸ provided genomic evidence of somatic hybridisation in *Pgt*, shedding light on the origin of Ug99 through the exchange of nuclei between standard race 21 and an unknown race. This is an important discovery to understand the formation of new diversity in the absence of sexual recombination.

The current South African *Pt* population consists of two primary genetic lineages²⁰, but at least five were evident according to *Pt* isolates collected during the previous century⁴⁹. Three of these appear to be extinct while only one lineage is expanding.^{27,28,30} Similar to *Pgt*, these new races probably represent exotic introductions as races with similar phenotypes and genotypes were found in countries to the north of South Africa.²³ Globally, the South African *Pt* races grouped significantly with isolates from the Middle East, Pakistan and New Zealand.⁵⁰

Based on microsatellite analysis, the four *Pst* races described in South Africa represent a single, clonal lineage.¹⁹ As opposed to these races, the recently identified *Pst* race in Zimbabwe was genetically very similar to two Kenyan isolates⁴⁵, indicating a southerly expansion of stripe rust diversity in Africa.

Due to the unique ability of markers to distinguish genotypes independently of their associated phenotypes, genetic screening of field isolates can detect variants before a new phenotype becomes evident. While *Pt* races 3SA38, 3SA10 and 3SA248 were first detected as phenotypic variants in 2016²⁷, their unique genotypes were already abundant in field isolates collected in 2015⁴⁹. These markers also indicated that within each phenotype, significant genetic variation was present, making genetic markers an effective supplementary tool to race phenotyping.

Host resistance

Resistance phenotypes in wheat are typically growth stage mediated. All-stage resistance (ASR), conferred by major genes, is clearly expressed

throughout the lifespan of the plant whereas adult plant resistance (APR), often polygenic and partial in manifestation, becomes effective at more mature growth stages.⁵¹ Phenotypes commonly encountered on adult plants are shown in Figure 1. As some APR genes are considered durable, this resistance type is frequently preferred in breeding and selection. Wheat cultivars carrying the pleiotropic race non-specific APR genes *Sr2/Yr30*, *Lr34/Yr18/Sr57*, *Lr46/Yr29/Sr58* and *Lr67/Yr46/Sr55* have maintained moderate levels of rust resistance under epidemic field trial conditions in South Africa and might not provide adequate protection when deployed singly under high disease pressure. Soko et al.⁵² recorded grain yield losses due to stem rust of between 10.1% and 19.5% for APR cultivars as opposed to a 6.4% loss in an ASR line. Previously, Pretorius et al.⁸ mentioned losses as high as 65% for susceptible wheat cultivars infected with stripe rust and a 56% yield gain was obtained when leaf rust was controlled by fungicide application on a susceptible cultivar. Breeders are therefore encouraged to either combine APR sources or stack them with ASR genes, the latter especially in areas prone to early-season infection.

The damage potential of wheat rusts is a reality, and it remains important to verify the resistance status of local germplasm and embark on appropriate breeding and selection programmes. As part of risk assessment and compilation of production guidelines, all commercially recommended wheat cultivars in South Africa, as well as leading breeding lines, are tested annually against a panel of rust races. These tests comprise seedling assays for ASR and field tests under high inoculum pressure in carefully managed rust nurseries. The University of the Free State has implemented rust nurseries with great success at the research facilities of Corteva Agriscience™ at Greytown in KwaZulu-Natal since the early 1990s. The Greytown environment is highly conducive to the vigorous development of both spring and winter wheat types as well as rust development. In a typical year, stripe rust would be first to establish during the cooler months of August and September, followed by leaf rust in October and finally stem rust, which peaks at the end of the season.

Stem rust assessments for local germplasm are summarised in Figure 2. Only cultivars with seedling infection types <2 (0 to 4 scale)⁵³, and a coefficient of infection⁵⁴ <20, were considered to carry true ASR. Some cultivars regarded as resistant as seedlings showed an intermediate stem rust reaction in the field and were thus not classified as displaying true ASR. Inoculum loads in the Greytown field nursery are extremely high and not all ASR genes provide complete rust protection under such conditions. In most cases it is assumed that these cultivars will be acceptable in commercial fields where inoculum pressure is lower. The opposite was also observed where some cultivars were classified as intermediate in the seedling stage but stem rust resistant in the field. The effect of using race PTKST in the field from 2011 onwards is clear from the initial decrease in resistance before a gradual improvement in resistant entries as breeders adapted their selection and breeding strategies. Collectively such information, also for leaf and stripe rust, adds to an understanding of disease risk and management at production level. To support field data, protocols for accelerated and reliable greenhouse assays have been developed for stripe rust^{16,55,56}, leaf rust⁵⁷ and stem rust⁵⁸.

Genetic studies of host resistance provide information on the monogenic or polygenic nature of genes involved, their identity and chromosome location, association with known genes or quantitative trait loci (QTL), and molecular markers for tracking the resistance. Together this knowledge contributes to assumptions of durability and targeted attempts to achieve long-lasting resistance. Ramburan et al.¹⁶ were the first to map rust resistance in a South African wheat cultivar. They identified three major stripe rust resistance loci in the spring wheat cultivar Kariega and paved the way for fine mapping and marker development for *QYr.sgi-2B.1* and *QYr.sgi-4A.1*, and confirmation of the pleiotropic resistance gene *Lr34/Yr18/Sr57*.^{57,59} In a similar approach, the durable stripe rust resistance of the European wheat cultivar Cappelle Desprez was mapped¹⁷ with subsequent identification of the major effect QTL *QYr.ufs-2A* along with three QTL of smaller effect, *QYr.ufs-2D*, *QYr.ufs-5B* and *QYr.ufs-6D*. Using histological techniques, Maree et al.^{60,61} investigated fungal behaviour in lines containing different combinations of the stripe rust resistance

QTL characterised in Kariega and Cappelle Desprez, respectively. These studies confirmed the value of gene stacking and careful selection of lines with the best ability to mitigate fungal invasion.

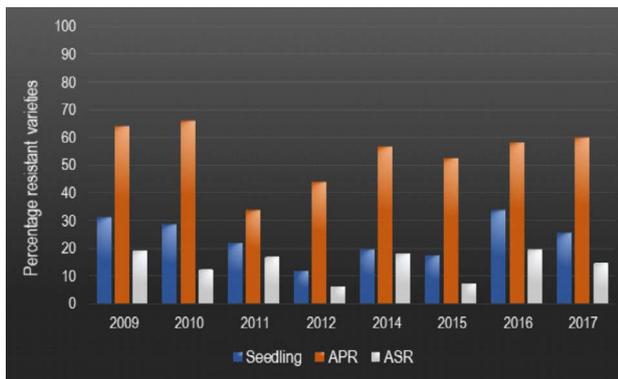


Figure 2: The frequency of South African wheat varieties expressing a low seedling response, adult plant resistance (APR) and true all-stage resistance (ASR) to stem rust over 8 years. In 2009 and 2010, entries were tested with *Puccinia graminis* f. sp. *tritici* pathotype UVPgt59 (TTKSP), and since 2011 with the more virulent UVPgt60 (PTKST) pathotype.

Prins et al.⁶² assessed stem rust response in an African wheat collection and identified several marker-trait associations in a genome-wide study. Two lines with exceptional APR were identified and biparental mapping populations developed. Marker-trait associations on chromosomes 6AS and 3BS and the *Lr34/Yr18/Sr57/Pm38* resistance locus were confirmed, along with stem rust resistance QTL not detected in the association study, one of which was the significant QTL *QSr.ufs-4D*. This emphasises the value of applying multiple approaches to unravel host resistance, particularly in cases where marker coverage in certain chromosomal areas is too low to detect QTL.

The availability of *Pgt* races with virulence attributes appropriate for targeting certain sources of resistance has contributed to several studies. These projects addressed phenotyping and genetics of resistance to Ug99 races⁶³⁻⁶⁶, resistance characterisation of triticale⁶⁷ and lines with genes transferred from *Aegilops sharonensis*⁶⁸ and *Thinopyrum ponticum*^{69,70}. Furthermore, Pretorius et al.⁷¹ demonstrated the application of remote sensing and the normalised difference vegetation index in reliably phenotyping wheat stripe rust response in the field.

Breeding and selection

Marker-assisted selection (MAS) is widely accepted as a key strategy to pyramid resistance genes into wheat genotypes, in particular, genes that do not exhibit easily distinguishable phenotypes.⁷² In South Africa, large-scale MAS was not implemented by breeding companies in the early 2000s⁸, although it was routinely used to select for several traits in countries such as Australia, Mexico, USA, the UK and India⁷². In 2011, a proposal by CenGen (Pty) Ltd. titled 'Establishment of a molecular marker service laboratory for routine application of marker-assisted selection in South African wheat breeding programs' (WCT/W/2009/02), was approved for funding by the Winter Cereal Trust. The capital expense of establishing a MAS laboratory and routine maintenance justified a central facility at CenGen for all wheat breeding programmes. The project is based on (1) purity testing of donor lines and confirmation of the target trait, (2) planning of breeding schemes and crosses to transfer the new trait, and (3) tracking the trait in subsequent filial generations.

South African seed companies use different strategies to breed for rust resistance, dependent on their approach, resources and location. Yet there is a collective focus on pyramiding rust resistance genes, in particular those that confer durable APR, to uphold the international drive of gene stewardship. Sensako (Pty) Ltd., a private breeding company with headquarters in Bethlehem (Free State, South Africa), follows a strategy in which they combine target genes/QTL in doubled haploid donor lines. This is followed by a top cross with their elite lines

or commercial cultivars and from the F_1 -generation doubled haploid lines are developed to integrate the genes/QTL into better adapted backgrounds. This approach has proven to be successful in pyramiding rust resistance genes/QTL (Figure 3). They have managed to develop a line containing multiple genes for resistance to all three rust pathogens, which is now used as a key donor line to incorporate complex resistance into existing cultivars.

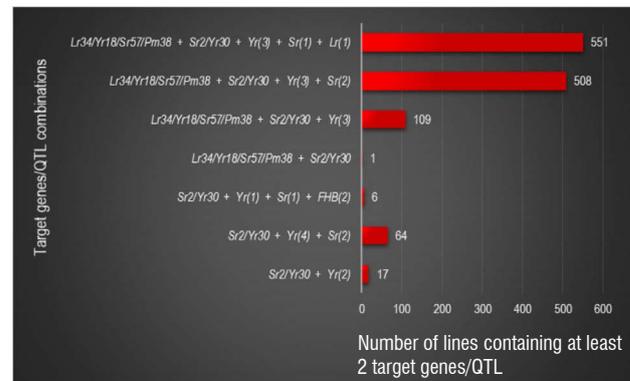


Figure 3: Complement of rust resistances incorporated in the Sensako doubled haploid marker-assisted selection programme. Numbers in brackets indicate the maximum number of genes/quantitative trait loci (QTL) screened for in the specific combination. For each combination, lines containing at least two target genes/QTL were selected for field evaluation in 2019. For all combinations, at least one line was recovered containing all targets.

The South African wheat breeding programme of Corteva Agriscience™ follows a more traditional approach of gradually incorporating multiple genes/QTL into their breeding lines. Gene enrichment is done at the F_2 -generation, and the presence of the target genes is confirmed in the F_5 -generation after three cycles of selection for agronomic traits. Pure lines containing the target genes are then either used to generate new resistance gene combinations or, if within the tolerance levels set for the different milling and baking quality criteria, are considered for commercial release. This programme has been successful in combining APR genes for stem, stripe and leaf rust resistance into elite breeding material that performs similarly to current commercial cultivars in yield trials (Table 1).

Table 1: Yield performance of selected marker-assisted selection (MAS) lines of Corteva Agriscience™ compared to commercial cultivars

Entry	Relative yield (%) ^a	Genes incorporated through MAS
Cultivar 01	102	Confidential ^b
Cultivar 02	97	Confidential
Cultivar 03	94	Confidential
Cultivar 04	100	Confidential
Cultivar 05	106	Confidential
Cultivar 06	106	Confidential
MAS Line 01	99	<i>Fhb1 Qfhs.ndsu-3BS; FHB Qfs.ifa-5A</i>
MAS Line 02	96	<i>Fhb1 Qfhs.ndsu-3BS; FHB Qfs.ifa-5A</i>
MAS Line 03	98	<i>Fhb1 Qfhs.ndsu-3BS; FHB Qfs.ifa-5A</i>
MAS Line 04	99	<i>Fhb1 Qfhs.ndsu-3BS; FHB Qfs.ifa-5A</i>
MAS Line 05	96	<i>Fhb1 Qfhs.ndsu-3BS; FHB Qfs.ifa-5A</i>
MAS Line 06	102	<i>Fhb1 Qfhs.ndsu-3BS; FHB Qfs.ifa-5A</i>
MAS Line 07	95	<i>Sr2/Yr30; Lr34/Yr18/Sr57/Pm38</i>

^aYields measured in tons/ha are expressed relative to Cultivar 04 which was taken as the benchmark (100%).

^bDeveloped through traditional breeding without MAS.

The MAS programme commenced in 2011 with the capacity to screen for 19 genes/QTL, of which 13 were related to rust resistance. These targets included the popular APR genes *Lr34/Yr18/Sr57/Pm38* (*Pm* is the notation for powdery mildew resistance genes) and *Sr2/Yr30*, the leaf rust resistance gene *Lr19*⁷³ as well as QTL previously identified for stripe rust resistance in the cultivars Kariega¹⁶ and Cappelle-Desprez¹⁷. Since its inception, the programme has grown to include 63 genes/QTL of which 29 are associated with rust resistance (Figure 4). These are obtained by breeders through international collaboration with organisations such as CIMMYT, or are newly identified sources from ongoing local research projects.⁶²

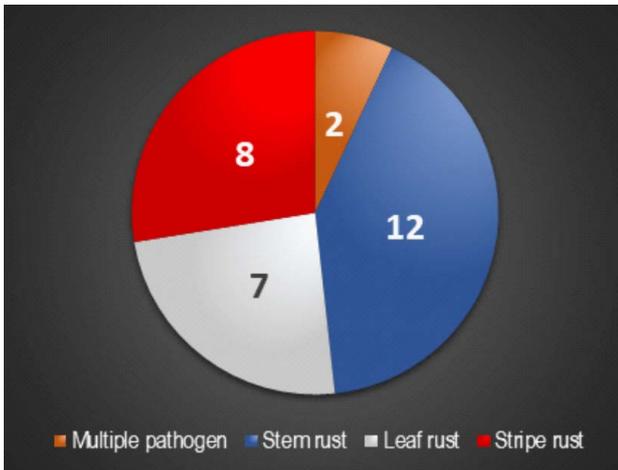


Figure 4: Targets of rust resistance genes screened for in the marker-assisted selection programme at CenGen.

Molecular markers for target genes/QTL are obtained from the public domain and research articles, or from in-house mapping projects by CenGen and collaborators. These include simple sequence repeat (SSR), sequence-tagged site (STS), cleaved amplified polymorphic site (CAPS) and single nucleotide polymorphism (SNP) markers. Since 2013, the implementation and upgrade of KASP™ SNPLINE™ instruments (LGC, UK) at CenGen greatly enhanced high-throughput capacity. The number of data points (calculated as the number of samples x number of markers tested per sample) that are generated annually continues to increase (Figure 5) despite a decrease in industry funding.

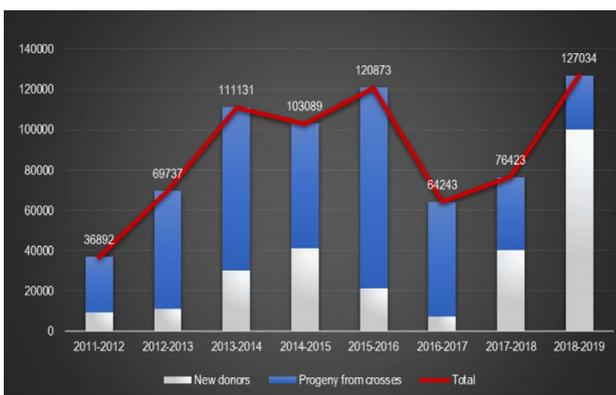


Figure 5: Number of data points generated since inception of the marker-assisted selection programme.

Notwithstanding the success of the implementation and application of the MAS programme for single locus traits such as rust resistance, the status of MAS in South Africa trails behind that of international programmes, which are exploring an integrated genomics-assisted breeding approach.⁷⁴ In 2010, crop geneticists started to investigate genomic selection in wheat to select for complex, multi-locus traits.⁷⁵ By 2012, reports of the value of genomic selection using genotyping-

by-sequencing in wheat were published, creating yet another avenue for genomics-assisted breeding (Figure 6).⁷⁶ The challenge remains for South African breeders and geneticists to follow international trends in genomics-assisted breeding and sensibly implement selection strategies for multi-locus traits.



MAS, marker-assisted selection; GS, genomic selection; GBS, genotyping by sequencing

Figure 6: Timeline of MAS in South Africa (bottom) compared to international programmes (top).

Conclusions

The relatively frequent introduction of new rust races into South Africa strongly suggests the possibility of further incursions. Stem rust and stripe rust, in particular, are extremely damaging diseases and the description of highly virulent and aggressive *Pst* and *Pgt* races in other wheat regions^{77,78} emphasises continued vigilance. The introduction of such races could impact severely on cultivar response with a consequent increase in production risk and cost. The survival of rust on off-season wheat crops and ancillary hosts such as wild rye (*Secale strictum* subsp. *africanum*) in the Roggeveld Mountains of the southwestern Karoo⁷⁹, requires further attention. Although samples collected from wild rye revealed *Pst*, the stem and leaf rust forms were those of cultivated rye and not bread wheat.⁷⁹ Wild rye is, however, moderately susceptible to *Pgt* and could serve as an inoculum source. The occurrence of both *Pt* and *Pgt* on a summer wheat crop in the eastern Free State in January 2020 (WHP Boshoff, unpublished) is of concern and supports the expansion of surveys to this period. Scientists should thus continue with surveillance, studies of pathogen variability, characterisation of cultivars, genetic analyses, resistance discovery, focused breeding and selection, and communication of research outcomes to producers. Overarching activities include international, regional and national collaboration; capacity building and training; embracing of new technologies; resistance gene stewardship; and sourcing sustained funding.

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Competing interests

We declare that there are no competing interests.

Authors' contributions

Z.A.P. developed the outline, wrote the Abstract, Background, 'Host resistance' section and Conclusions; R.P. and E.W. wrote the 'Breeding and selection' section including table and figures; C.M.B. provided long-term cultivar data; B.V. wrote 'Genetic analysis of *Puccinia* isolates'; W.H.P.B. wrote 'Rust surveillance and phenotypic analysis'. All authors contributed to editing of the final manuscript. Z.A.P. and W.H.P.B. provided photographs of rust phenotypes.

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