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# Distribution of *Puccinia striiformis* f. sp. *tritici* Races and Virulence in Wheat Growing Regions of Kenya from 1970 to 2014

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## Abstract

Stripe rust, caused by the fungal pathogen *Puccinia striiformis* f. sp. *tritici*, is a major threat to wheat (*Triticum* spp.) production worldwide. The objective of this study was to determine the virulence of *P. striiformis* f. sp. *tritici* races prevalent in the main wheat growing regions of Kenya, which includes Mt. Kenya, Eastern Kenya, and the Rift Valley (Central, Southern, and Northern Rift). Fifty *P. striiformis* f. sp. *tritici* isolates collected from 1970 to 1992 and from 2009 to 2014 were virulence phenotyped with stripe rust differential sets, and 45 isolates were genotyped with sequence characterized amplified region (SCAR) markers to differentiate the isolates and identify aggressive strains *PstS1* and *PstS2*. Virulence corresponding to stripe rust resistance genes *Yr1*, *Yr2*, *Yr3*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr25*, and *Yr27* and the seedling resistance in genotype Avocet S were detected. Ten races were detected in the *P. striiformis* f. sp. *tritici* samples obtained from 1970 to 1992, and three additional races were detected from 2009 to 2014, with a single race being detected in both periods. The SCAR markers detected both *Pst1* and *Pst2* strains in the collection. Increasing *P. striiformis* f. sp. *tritici* virulence was found in the Kenyan *P. striiformis* f. sp. *tritici* population, and different *P. striiformis* f. sp. *tritici* race groups were found to dominate different wheat growing regions. Moreover, recent *P. striiformis* f. sp. *tritici* races in East Africa indicated possible migration of some race groups into Kenya from other regions. This study is important in elucidating *P. striiformis* f. sp. *tritici* evolution and virulence diversity and useful in breeding wheat cultivars with effective resistance to stripe rust.

*Keywords*: cereals and grains, field crops, fungi, pathogen diversity, pathogenicity, *Puccinia* f. sp. *tritici*, stripe (yellow) rust, *Triticum aestivum*

Stripe (yellow) rust, caused by the fungal pathogen *Puccinia striiformis* Westend. f. sp. *tritici* Erikss., is an important disease of wheat (*Triticum* spp.) causing significant yield losses worldwide (Hovmøller et al. 2011). In Kenya, stripe rust was reported as early as 1908 (Leonard 2001; Thorpe 1959; Wanyera 1994). The disease is found in all Kenyan wheat growing areas but is most prevalent in the Rift Valley regions. Stripe rust reduces both grain quality and yield (Wellings 2011), with yield losses of ≤100% being reported in susceptible wheat varieties (Chen 2005; Leonard 2001; Thorpe 1959; Wanyera 1994; Wellings 2007). In East Africa (Kenya and Ethiopia), virulence to stripe rust resistance genes *Yr9* and *Yr27* resulted in yield losses of ≤40% in commercial varieties such as 'Paa', which carries *Yr9* (Danial et al. 1994; Saari and Prescott 1985). Although the impact of stripe rust on yields has been evident in the last several decades, no significant epidemics have been reported in Kenya.

Pathogenic variation within *P. striiformis* f. sp. *tritici* was first demonstrated by Gassner and Straib (1932). The wheat–*P. striiformis* f. sp. *tritici* pathosystem follows the gene-for-gene concept (Flor 1942). Currently, >85 genes conferring resistance to *P. striiformis* f. sp. *tritici* have been formally designated, and >100 quantitative trait loci have been reported (McIntosh et al. 2017; Wang et al. 2017). Most of these stripe rust resistance genes confer all stage resistance, being effective throughout the life of the wheat plant. However, many of these genes are rendered ineffective in a short span of time, given the evolution for virulence to specific resistance genes within individual *P. striiformis* f. sp. *tritici* isolates as a result of mutation in the corresponding avirulence genes.

Races of *P. striiformis* f. sp. *tritici* are identified by their infection types (ITs) on resistance genes defined in a set of wheat lines and near isogenic lines used as differentials. *P. striiformis* f. sp. *tritici* race surveys are undertaken in many countries, which involve greenhouse testing of *P. striiformis* f. sp. *tritici* isolates on seedlings of these differential wheat genotypes (Pretorius et al. 2017). These in-country surveys are essential for following changes in *P. striiformis* f. sp. *tritici* virulence profiles relative to the wheat varieties grown. Information on virulence for specific resistance genes and virulence diversity helps breeders to deploy effective resistance genes in breeding new wheat varieties.

The epidemiology of stripe rust is influenced not only by the genes deployed in wheat varieties but also by climatic conditions that affect *P. striiformis* f. sp. *tritici* infection and growth and by wind movements across areas where wheat is grown (Chen 2005). *P. striiformis* f. sp. *tritici* urediniospores are dispersed primarily by wind, but accidental transmission by humans has also been reported. For example, *P. striiformis* f. sp. *tritici* was introduced from northwestern Europe to Australia in 1979 (Hovmøller et al. 2008, 2011; Wellings 1988, 2007). However, the capacity for long-distance dispersal of *P. striiformis* f. sp. *tritici* is essential for the incursion of new races. For instance, virulence for *Yr9*, found in Ethiopia in 1986, spread to the Middle East and South Asia in a single decade (Hovmøller et al. 2008; Singh et al. 2004). Likewise, stripe rust reported in South Africa in 1996 was probably dispersed by wind from North and East Africa (Boshoff et al. 2002).

Amplified fragment length polymorphism and sequence characterized amplified region (SCAR) markers have been used to characterize *P. striiformis* f. sp. *tritici* isolates to understand their evolution and define genetic variation of the pathogen (Justesen et al. 2002;

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Wellings et al. 2004). Based on amplified fragment length polymorphism markers distinguishing the two closely related aggressive and high-temperature adapted *P. striiformis* f. sp. *tritici* strains *PstS1* and *PstS2*, SCAR markers were developed and applied on a collection of 566 world isolates (Walter et al. 2016). *PstS1* was found in East Africa in the early 1980s and in the Americas and Australia in 2000 and 2002, respectively (Ali et al. 2017; Brar et al. 2018; Walter et al. 2016). *PstS2*, thought to be derived from *PstS1*, was widespread in the Middle East and Central Asia and was detected in Europe in 2000, although it was never widespread in the European *P. striiformis* f. sp. *tritici* population. *PstS1* and *PstS2* can both tolerate high temperatures and therefore are often found in warm areas of the world and can spread into areas where stripe rust was not previously detected (Ali et al. 2017; Milus et al. 2009; Walter et al. 2016).

The objective of this study was to determine the avirulence or virulence profiles of *P. striiformis* f. sp. *tritici* isolates collected in Kenya in two periods, 2009 to 2014 and 1970 to 1992, the latter based on the Kenyan isolates in the historic Stubbs collection (Thach et al. 2015). These two collections from Kenya were compared through the use of SCAR markers to distinguish between *P. striiformis* f. sp. *tritici* strains *PstS1* and *PstS2*.

## Materials and Methods

**Wheat genotypes used to determine *P. striiformis* f. sp. *tritici* races.** Nineteen stripe rust differential wheat genotypes, each containing a specific resistance genes, were from the Global Rust Research Centre, Denmark (Table 1). These included six near-isogenic lines in the 'Avocet' background (Hovmøller et al. 2017), five world differentials ('Chinese 166', 'Vilmorin 23', 'Heines Kolben', 'Lee', and 'Moro'), three European differentials ('Hybrid 46', 'VPM1', and 'Carstens V'), and five wheat genotypes ('Kalyansona', 'Cortez', 'TP981', 'Opata', and 'Ambition'). The wheat genotype 'Cartago' was included as a susceptible control.

***P. striiformis* f. sp. *tritici* isolates.** A total of 50 *P. striiformis* f. sp. *tritici* isolates (Table 2) were collected, including 37 from 1970 to 1992 in the Stubbs collection (Thach et al. 2015) and 13 from wheat growing regions in Kenya in 2009 to 2014. All 50 isolates were phenotyped for virulence, and 45 of them were genotyped with the SCAR markers reported by Walter et al. (2016).

**Table 1.** Wheat varieties with known yellow rust (*Yr*) genes used for virulence analysis of *Puccinia striiformis* f. sp. *tritici* Kenyan isolates collected from 1970 to 1992 and from 2009 to 2014

| Wheat differentials[a] | *Yr* genes[b] | Seed source | Differential type or grouping |
|---|---|---|---|
| Cartago | None | Flak 10 | Control |
| Chinese 166 | *Yr1* | Flak 10 | World |
| Kalyansona | *Yr2*, + | Flak 08 | Others |
| Vilmorin 23 | *Yr3*, + | Flak 06 | World |
| Hybrid 46 | *Yr4*, + | Flak 07 | European |
| Heines Kolben | *Yr6*, + | Flak 06 | World |
| Avocet *Yr6* | *Yr6*, *YrAvS* | Flak 09 | Avocet NIL[c] |
| Lee | *Yr7*, + | Flak 09 | World |
| Avocet *Yr8* | *Yr8* | Flak 09 | Avocet NIL |
| Avocet *Yr9* | *Yr9*, *YrAvS* | Flak 12 | Avocet NIL |
| Moro | *Yr10*, + | Flak 09 | World |
| Cortez | *Yr15* | Flak 09 | Others |
| VPM1 | *Yr17*, + | Flak 12 | European |
| Avocet *Yr17* | *Yr17*, *YrAvS* | Flak 09 | Avocet NIL |
| TP981 | *Yr25*, + | Flak 09 | Others |
| Opata | *Yr27*, *Yr18*, + | Flak 10 | Others |
| Carstens V | *Yr32*, *Yr25*, + | Flak 06 | European |
| Avocet S | *YrAvS* | Flak 12 | Avocet NIL |
| Ambition | *YrAmb* | Flak 12 | Others |
| Avocet *Yr5b*[d] | *Yr5b*, *Yr18*, *YrAvS* | Flak 13 | Avocet NIL |

[a] Seed was subsequently multiplied at the National Institute of Agricultural Botany.
[b] References for *Yr* genes and interpretation of infection types in McIntosh et al. (1995) and Hovmøller and Justesen (2007).
[c] NIL, near isogenic lines. The Avocet NILs were developed by Wellings et al. (2004).
[d] The gene *Yr5b* corresponds to the previously defined *YrSP* locus as the cloning of these genes found that *Yr5a* (previously *Yr5*) and *Yr5b* (previously *YrSP*) are allelic (Marchal et al. 2018).

**Spore multiplication and purification.** Wheat genotypes 'Cartago' and 'Morocco' were sown in 6.5 × 6.5 × 7 cm plastic pots filled with peat moss (Unimuld, Ryomgaard, Denmark) and placed in a 35 × 44 cm plastic tray in spore-proof greenhouse cabins, maintained on a 17°C day/12°C night cycle. Two milliliters of maleic hydrazine (1,2-Dihydro-3,6-pyridazinedione, 0.25 g/liter) solution (Antergon, MH 180, Crompton Registrations Ltd., Birmingham, England) was added to each pot when the seedlings were approximately 2 cm to slow plant growth and increase *P. striiformis* f. sp. *tritici* spore production.

Seedlings were inoculated with *P. striiformis* f. sp. *tritici* isolates when they were 10 to 12 days old (Feekes stage 1) (Large 1954). Urediniospores of the *P. striiformis* f. sp. *tritici* isolates were retrieved from liquid nitrogen (−196°C) and heat-shocked in a water bath at 40°C for 2 min to break dormancy. We poured the spores into a glassine bag and inoculated the plants by rubbing spores onto the seedlings (Hovmøller et al. 2017). We maintained aseptic conditions between isolate inoculations by rinsing the fume hood and equipment with 70% ethanol. We maintained relative humidity (RH) at 100% by misting plants with water with a hand sprayer, and then we covered the inoculated seedlings with a lid before transferring them to an incubation dew chamber at 10°C for 24 h in total darkness. After 24 h, the inoculated seedlings were transferred to spore-proof greenhouse cabins and kept under the same conditions as described below. The seedlings were covered with cellophane bags (Helmut Schmidt Verpackungsfolien GmbH, Königswinter, Germany) 7 days after inoculation (DAI), before lesions had begun to appear on the plants. Approximately 15 to 20 DAI, we collected spores by gently shaking them off the seedlings and transferring them into cryo vials. Spores were dried in a desiccator containing silica gel at room temperature for 4 days and subsequently stored in liquid nitrogen at −196°C.

**Determination of *P. striiformis* f. sp. *tritici* races.** *P. striiformis* f. sp. *tritici* races were determined from 19 wheat stripe rust differential lines (Table 1; Hovmøller et al. 2017; Thach et al. 2015). Differential lines at Feekes stage 1 were inoculated with each *P. striiformis* f. sp. *tritici* isolate as a spore suspension in 3M Novec$^{T}$ 7100 fluid via the airbrush spray gun method (Thach et al. 2015). Inoculated seedlings were then incubated in a dew chamber at approximately 100% RH and 10°C for 24 h in darkness. The inoculated plants were thereafter transferred to spore-proof greenhouse cabins and maintained at 17°C day/12°C night with cycles of 16 h light and 8 h darkness and supplemented with artificial light of 200 µmol/s/m, 70 to 80% RH.

Stripe rust infection on first and second seedling leaves was evaluated approximately 16 DAI on a 0 to 9 scale (McNeal et al. 1971). The race of each *P. striiformis* f. sp. *tritici* isolate was determined based on its IT on each differential, with IT scores between 0 and 6 being considered incompatible, whereas ITs 7 to 9 were considered compatible (Hovmøller et al. 2017). Races were numbered according to the occurrence of virulence to the *Yr* genes included in the virulence test following the order indicated in Table 1.

We purified six isolates that appeared to be a mixture of races by isolating single lesion and growing them on the key differential genotypes 'Avocet *Yr8*', 'TP981', 'Opata', 'Vilmorin 23', 'VPM1', and 'Heines VII'. A single lesion was collected from the key differential genotypes, and race testing was repeated. The isolates that were purified included KE78107 (*Yr8*), KE78107 (TP981), KE92011 (Opata), KE92011 (VPM1), KE23/09 (V23), and KE23/09 (H.VII).

**DNA extraction and SCAR marker testing.** Forty-five of the 50 *P. striiformis* f. sp. *tritici* isolates were genotyped with two SCAR markers, SCP19M24 and SCP12M26 (Walter et al. 2016). Genomic DNA was extracted with the sbeadex mini plant kit (LGC Genomics GmbH, Germany) according to the manufacturer's instructions and automated with a KingFisher Magnetic Particle Processor (Thermo Fisher Scientific, USA). Approximately 3 mg of urediniospores of each isolate were ground with two steel balls in a Geno/Grinder 2010 (SPEX SamplePrep, USA) at 1,500 × *g* for 90 s.

**Table 2.** Virulence analysis of *Puccinia striiformis* f. sp. *tritici* isolates collected in Kenya between 1970 and 2014

| Race no. | Virulence profile | Isolate | Year of collection | Location | Coordinates | Elevation (m) | Region |
|---|---|---|---|---|---|---|---|
| 1 | *Yr2*, *Yr6*, *Yr7*, *YrAvS* | KE78105 | 1978 | Ngongongeri | 0.3500°S, 35.9167°E | 2,300 | Central Rift |
| | | KE89009 | 1989 | Njoro | 0.3290°S, 35.9440°E | 2,174 | Central Rift |
| 2 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *YrAvS* | KE74195 | 1974 | Molo | 0.2479°S, 35.7374 °E | 2,534 | Central Rift |
| | | KE76004 | 1976 | Njoro | 0.3290°S, 35.9440°E | 2,174 | Central Rift |
| | | KE78019 | 1978 | Njoro | 0.3290°S, 35.9440°E | 2,174 | Central Rift |
| | | KE78102 | 1978 | Makutano Nakuru | 1.2577°N, 35.0927°E | 2,050 | Central Rift |
| | | KE78107 *Yr8* | 1978 | Eldoret | 0.5204°N, 35.2699°E | 2,062 | Northern Rift |
| | | KE81010 | 1981 | Njoro | 0.3290°S, 35.9440°E | 2,174 | Central Rift |
| | | KE81046 | 1981 | Subukia | 0.0062°S, 36.1733°E | 2,012 | Central Rift |
| | | KE81111 | 1981 | Mununguwa | 1.3833°S, 37.2500°E | 1,132 | Eastern |
| | | KE81082 | 1981 | Kaptagat | 0.4333°N, 35.4833°E | 2,337 | Northern Rift |
| | | KE81086 | 1981 | Molo | 0.2479°S, 35.7374°E | 2,534 | Central Rift |
| | | KE82017 | 1982 | Mt. Kenya | 0.4500°S, 37.1168°E | 2,238 | Mt. Kenya |
| | | KE82044 | 1982 | Endebess | 1.0740°N, 34.8565°E | 1,883 | Northern Rift |
| | | KE86063 | 1986 | Njoro | 0.3290°S, 35.9440°E | 2,174 | Central Rift |
| | | KE87035 | 1987 | Ngorengore | 1.0333°S, 35.5000°E | 2,029 | Southern Rift |
| | | KE87048 | 1987 | Mau Narok | 0.5996°S, 36.0069°E | 2,825 | Central Rift |
| | | KE87071 | 1987 | Njoro | 0.3290°S, 35.9440°E | 2,174 | Central Rift |
| | | KE89008 | 1989 | Njoro | 0.3290°S, 35.9440°E | 2,174 | Central Rift |
| 3 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr25*, *YrAvS* | KE82046 | 1982 | Molo | 0.2479°S, 35.7374°E | 2,534 | Central Rift |
| 4 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr27*, *YrAvS* | KE79001 | 1979 | Molo | 0.2479°S, 35.7374°E | 2,534 | Central Rift |
| | | KE81070 | 1981 | Maralal | 1.0968°N, 36.6980°E | 1,811 | Northern Rift |
| | | KE81040 | 1981 | Mt. Kenya | 0.4500°S, 37.1168°E | 2,238 | Mt. Kenya |
| | | KE82026 | 1982 | Marania farm | 0.0333°N, 37.5500°E | 2,371 | Mt. Kenya |
| | | KE82031 | 1982 | Maralal | 1.0967°N, 36.6979°E | 1,811 | Northern Rift |
| | | KE82028 | 1982 | Ngorengore | 1.0333°S, 35.5000°E | 2,029 | Southern Rift |
| | | KE82029 | 1982 | Embori | 0.0652°N, 37.3487°E | 2,636 | Mt. Kenya |
| | | KE90083 | 1990 | Mau Narok | 0.5996°S, 36.0069°E | 2,825 | Central Rift |
| 5 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr25*, *Yr27*, *YrAvS* | KE70063 | 1970 | Molo | 0.2479°S, 35.7374°E | 2,534 | Central Rift |
| | | KE82043 | 1982 | Eldoret | 0.5203°N, 35.2699°E | 2,062 | Northern Rift |
| | | KE328/13 | 2013 | KALRO, Njoro | 0.3410°S, 35.9450°E | 2,185 | Central Rift |
| 6 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *YrAvS* | KE90006 | 1990 | Njoro | 0.3290°S, 35.9440°E | 2,174 | Central Rift |
| 7 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr27*, *YrAvS* | KE92011 Opata | 1992 | Njoro | 0.3290°S, 35.9440°E | 2,174 | Central Rift |
| | | KE92011 VPM1 | 1992 | Njoro | 0.3290°S, 35.9440°E | 2,174 | Central Rift |
| 8 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27*, *YrAvS* | KE91013 | 1991 | Njoro | 0.3290°S, 35.9440°E | 2,174 | Central Rift |

(*Continued on next page*)

**Table 2.** (*Continued from previous page*)

| Race no. | Virulence profile | Isolate | Year of collection | Location | Coordinates | Elevation (m) | Region |
|---|---|---|---|---|---|---|---|
| 9 | *Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *YrAvS* | KE79067 | 1979 | Njoro | 0.3290°S, 35.9440°E | 2,174 | Central Rift |
| 10 | *Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr25*, *YrAvS* | KE80022 | 1980 | Njoro | 0.3290°S, 35.9440°E | 2,174 | Central Rift |
| 11 | *Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27*, *YrAvS* | KE17/09 | 2009 | KALRO, Njoro | 0.3410°S, 35.9450°E | 2,185 | Central Rift |
| | | KE18/09 | 2009 | KALRO, Njoro | 0.3410°S, 35.9450°E | 2,185 | Central Rift |
| | | KE19/09 | 2009 | KALRO, Njoro | 0.3410°S, 35.9450°E | 2,185 | Central Rift |
| | | KE23/09 H.VIII | 2009 | Njoro | 0.5204°N, 35.2699°E | 2,174 | Central Rift |
| | | KE126a/11 | 2011 | KALRO, Njoro | 0.3410°S, 35.9450°E | 2,185 | Central Rift |
| | | KE132b/11 | 2011 | Mathangauta | 0.5621°S, 35.9840°E | 2,585 | Central Rift |
| | | KE327/13 | 2013 | KALRO, Njoro | 0.3410°S, 35.9450 °E | 2,185 | Central Rift |
| | | KE127/14 | 2014 | KALRO, Njoro | 0.3410°S, 35.9450°E | 2,185 | Central Rift |
| | | KE129/14 | 2014 | KALRO, Njoro | 0.3410°S, 35.9450°E | 2,185 | Central Rift |
| | | KE131/14 | 2014 | KALRO, Njoro | 0.3410°S, 35.9450°E | 2,185 | Central Rift |
| 12 | *Yr1*, *Yr2*, *Yr3*, *Yr6*, *Yr9*, *Yr25*, *YrAvS* | KE78107 TP | 1978 | Eldoret | 0.5203°N, 35.2699°E | 2,062 | Northern Rift |
| 13 | *Yr2*, *Yr3*, *Yr6*, *Yr25*, *YrAvS* | KE23/09 V23 | 2009 | Njoro | 0.3431°S, 35.9479°E | 2,174 | Central Rift |
| 14 | *Yr2*, *Yr3*, *Yr6*, *Yr7*, *Yr8*, *Yr17*, *Yr25*, *YrAvS* | KE138b/11 | 2011 | Mailinne | 0.0260°N, 36.4262°E | 2,325 | Central Rift |

Each of the ground samples was incubated with 150 µl of lysis buffer PN and 1 µl of RNase (100 mg/ml) at 65°C for 10 min. The lysate (supernatant) was centrifuged at 1,500 × g for 10 min, and 50 µl of the lysate was used for DNA extraction. The DNA concentration was measured by spectrophotometry (Nanodrop 2000; Thermo Fisher Scientific, USA). The DNA stock solution was diluted to 30 ng/µl with sterilized double-distilled $H_2O$ and used as the working solution for PCR analyses. PCR was performed as described by Walter et al. (2016). The SCAR markers SCP19M24 and SCP12M26 from Walter et al. (2016) were validated and used in this study. The SCAR marker bands were scored as (+) for the presence and (−) for absence and allowed for the grouping of isolates into *PstS1*, *PstS2*, or others (neither *PstS1* nor *PstS2*) strains.

## Results

Fifty *P. striiformis* f. sp. *tritici* isolates, originating from the wheat growing areas of Kenya—Mt. Kenya, Eastern Kenya, and the Rift Valley (Central, Southern, and Northern Rift)—were tested in this study (Table 2). Seventy-four percent (37) of the isolates were retrieved from the Stubbs collection, and 26% (13) were collected from wheat fields across Kenya from 2009 to 2014. About 48% (24) of the isolates were collected from Njoro and the Kenya Agricultural and Livestock Research Organization (KALRO), Njoro Station (0.3290°S, 35.9440°E and 0.3410°S, 35.9450°E, respectively) in the Central Rift Valley (Table 2).

**Virulence phenotypes and races.** Virulence was detected for stripe rust resistance genes *Yr1*, *Yr2*, *Yr3*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr25*, and *Yr27* and the seedling resistance in 'Avocet S'. Virulence to *Yr4*, *Yr5b*, *Yr10*, *Yr15*, *Yr32*, and *YrAmb* were not detected. Fourteen *P. striiformis* f. sp. *tritici* races were identified from the 50 isolates (Tables 2 and 3). Ten races were detected in the Kenyan isolates from the Stubbs collection made from 1970 to 1992 and three from the isolates collected from 2009 to 2014. One race was detected in both periods (Table 3). Some of the races were virulent on as few as four *Yr* genes (*Yr2*, *Yr6*, *Yr7*, and *YrAvS*), and other races were virulent on up to nine *Yr* genes (*Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27*, and *YrAvS*) (Table 3). Virulence to *Yr2* and *Yr6* was found in both the old and new *P. striiformis* f. sp. *tritici* populations (Tables 2 and 3). Variation was observed in the frequency of virulence to *Yr1*, *Yr3*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, and *Yr27* (Fig. 1).

**Variability in *P. striiformis* f. sp. *tritici* races and virulence across the years of collection.** Looking at the number of virulence phenotypes in relation to year of collection, the isolates from 1970 to 1992 had fewer virulences (number of virulences per race ranged from four to eight) compared with the recently collected isolates (number of virulences per race ranged from five to nine) against the differential wheat genotypes used in this study (Table 3). Race 2 (with virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8*, and *YrAvS*) was the most prominent race, identified from 17 isolates from the Stubbs collection in 1974 to 1989. Race 4 (Race 2 + virulence to *Yr27*) was also prevalent in the Kenyan isolates of the Stubbs collection, identified from eight isolates collected over 4 years from 1979 to 1990. Among the recently collected *P. striiformis* f. sp. *tritici* isolates, Race 11 (Race 2 + *Yr1*, *Yr9*, *Yr25*, *Yr27*) was the most prominent, from 10 isolates collected in 2009 to 2014.

In 1978, three races, Race 1 (Race2–*Yr8*), Race 2 (*Yr2*, *Yr6*, *Yr7*, *Yr8*, and *YrAvS*), and Race 12 (*Yr1*, *Yr2*, *Yr3*, *Yr6*, *Yr9*, *Yr25*, and *YrAvS*), were detected. Two races were detected in 1979 (Races 4 and 9) and 1981 (Races 2 and 4). In 1982, four races, Race 2 (*Yr2*, *Yr6*, *Yr7*, *Yr8*, and *YrAvS*), Race 3 (Race 2 + *Yr25*), Race 4 (Race 2 + *Yr27*), and Race 5 (Race2 + *Yr25* + *Yr27*), were detected. Two races were identified in 1990 (Races 4 and 6), 2011 (Races 11 and 14), 2013 (Races 5 and 11), and 2014 (Race 11). In all other years of collection, only a single race was detected.

**Variability in *P. striiformis* f. sp. *tritici* races and virulence in wheat growing regions of Kenya.** The majority (72%) of *P. striiformis* f. sp. *tritici* isolates, in both the Stubbs collection and the newer isolates, were collected from the Central Rift Valley. Nearly all *P. striiformis* f. sp. *tritici* races were detected in this

region, the exception being Race 12, which was only found in the Northern Rift Valley. The prominent races in the Central Rift Valley were Race 2 (virulent on *Yr2*, *Yr6*, *Yr7*, *Yr8*, and *YrAvS*) and Race 11 (virulent on *Yr1*, *Yr2*, *Yr6*, *Yr8*, *Yr9*, *Yr25*, *Yr27*, and *YrAvS*), representing 34 and 20% of the total isolates, respectively. Race 2 isolates from the Central Rift Valley were all old isolates from the Stubbs collection, and Race 11 (from the same region) consisted of *P. striiformis* f. sp. *tritici* isolates collected from 2009 to 2014 (Fig. 2). Race 2 was found in all the five wheat growing regions of Kenya, but again in the old Stubbs collection.

Virulences to *Yr1*, *Yr9*, *Yr17*, and *Yr25* were detected only in the *P. striiformis* f. sp. *tritici* isolates collected from the Central and Northern Rift valleys (Table 2). Virulence to *Yr27* was found in all regions except the Eastern region. In addition, variation in *Yr* virulence was also observed within regions. In the Northern Rift Valley, virulence to *Yr1* was present in one *P. striiformis* f. sp. *tritici* isolate (KE78107 TP) collected from Eldoret but was absent in isolates from Kaptagat, Maralal, and Endebess. In the Central Rift Valley, virulence to *Yr1* was present in isolates from Njoro and KALRO Njoro and Mathangauta but absent in isolates from Ngongongeri, Molo, Makutano Nakuru, Subukia, Mau Narok, and Mailinne. Regional variation was also observed for virulence to *Yr9*, *Yr25*, and *Yr27* (Tables 2 and 3).

**Genotyping *P. striiformis* f. sp. *tritici* isolates by using SCAR markers.** SCAR markers SCP19M24 and SCP12M26 were used to determine whether the invasive strains *PstS1* and *PstS2* were present in the Kenyan *P. striiformis* f. sp. *tritici* isolates. SCP19M24 differentiated *PstS1* and *PstS2* strains from other strains, and SCP12M26 differentiated *PstS1* and *PstS2*. A total of 82% of the isolates were categorized as *PstS1*, and 86% of these isolates were collected from 1970 to 1992 (Table 4). Three isolates (6.6%), classified as *PstS2*, were collected in 2014 at KALRO Njoro (Table 4). These *PstS2* isolates all belonged to Race 13. Four isolates (8.8%) did not fall into either the *PstS1* or *PstS2* categories and were therefore considered other strains (Table 4, Fig. 3). These four isolates of other strains were represented by Races 5, 8, 11, and 12 and were collected in 1970, 1979, 2009, and 1978, respectively.

## Discussion

In this study, we considered the distribution of *P. striiformis* f. sp. *tritici* virulences and races in the wheat growing regions of Kenya, focusing on isolates collected between the two time frames of 1970 to 1992 and 2009 to 2014. Comparison of the *P. striiformis* f. sp. *tritici* isolates collected in this study (2009 to 2014) to the older isolates in the Stubbs collection indicated a trend toward increased virulence. However, most of the isolates collected between 2009 and 2014 were from the Njoro region, limiting the geographic diversity of the new *P. striiformis* f. sp. *tritici* collection. This limitation was caused primarily by the use of fungicides by informed farmers, limiting the number of fields that were infected with stripe rust. The race with the lowest number of *Yr* gene virulences, Race 1 (*Yr2*, *Yr6*, *Yr7*, and *YrAvS*), was first detected in 1978, and the race with the largest number of virulences was first detected in 2009 (Race 11 with virulence to *Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27*, and *YrAvS*). New races usually appear via stepwise mutation within the existing *P. striiformis* f. sp. *tritici* population, followed by selection caused by resistance genes in wheat cultivars, but sexual recombination, somatic hybridization, and incursion of new isolates, as seen in northwestern Europe with the emergence of the "Warrior" and "Kranich" races in 2011, may also contribute to the emergence of new races (Hovmøller et al. 2016; Hubbard et al. 2015). Sudden changes in *P. striiformis* f. sp. *tritici* races can result in stripe rust epidemics if the new races render the resistance genes deployed in currently grown wheat cultivars ineffective (Brar et al. 2018; Cheng et al. 2015; Jin et al. 2010; Lei et al. 2017; Singh et al. 2004; Stubbs 1988; Wellings 2011).

Whereas virulence to *Yr2*, *Yr6*, and *YrAvS* appeared to have become fixed in the *P. striiformis* f. sp. *tritici* population in Kenya as early as 1970, virulence for *Yr7* and *Yr8* was found to be increasing in frequency. Because the occurrence of virulence is attributed to the stripe rust resistance genes deployed in the wheat cultivars grown in the region (Walter et al. 2016), this finding suggests that *Yr2* and other genes have been deployed extensively in wheat cultivars grown in Kenya, whereas *Yr7* and *Yr8* have been deployed more recently. *Yr2* and *Yr6* are widespread in wheat varieties worldwide, and

**Table 3.** Races and genetic lineages of *Puccinia striiformis* f. sp. *tritici* isolates collected in Kenya between 1970 and 2014

| Race no. | Virulence phenotype | Number of virulences | Year detected | No. of isolates | Genetic lineage |
|---|---|---|---|---|---|
| 1 | *Yr2*, *Yr6*, *Yr7*, *YrAvS* | 4 | 1978; 1989 | 2 | *PstS1* |
| 2 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *YrAvS* | 5 | 1974; 1976; 1978; 1981; 1982; 1986; 1987; 1989 | 17 | *PstS1* |
| 3 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr25*, *YrAvS* | 6 | 1982 | 1 | *PstS1* |
| 4 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr27*, *YrAvS* | 6 | 1979; 1981; 1982; 1990 | 8 | *PstS1* |
| 5 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr25*, *Yr27*, *YrAvS* | 7 | 1970; 1982; 2013; | 3 | Others; *PstS1* |
| 6 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *YrAvS* | 7 | 1990 | 1 | *PstS1* |
| 7 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr27*, *YrAvS* | 7 | 1992 | 2 | *PstS1* |
| 8 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27*, *YrAvS* | 8 | 1991 | 1 | *PstS1* |
| 9 | *Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *YrAvS* | 6 | 1979 | 1 | Others |
| 10 | *Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr25*, *YrAvS* | 7 | 1980 | 1 | *PstS1* |
| 11 | *Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27*, *YrAvS* | 9 | 2009; 2011; 2013; 2014 | 10 | *PstS1*; *PstS2*; others |
| 12 | *Yr1*, *Yr2*, *Yr3*, *Yr6*, *Yr9*, *Yr25*, *YrAvS* | 7 | 1978 | 1 | Others |
| 13 | *Yr2*, *Yr3*, *Yr6*, *Yr25*, *YrAvS* | 5 | 2009 | 1 | *PstS2* |
| 14 | *Yr2*, *Yr3*, *Yr6*, *Yr7*, *Yr8*, *Yr17*, *Yr25*, *YrAvS* | 8 | 2011 | 1 | *PstS1* |

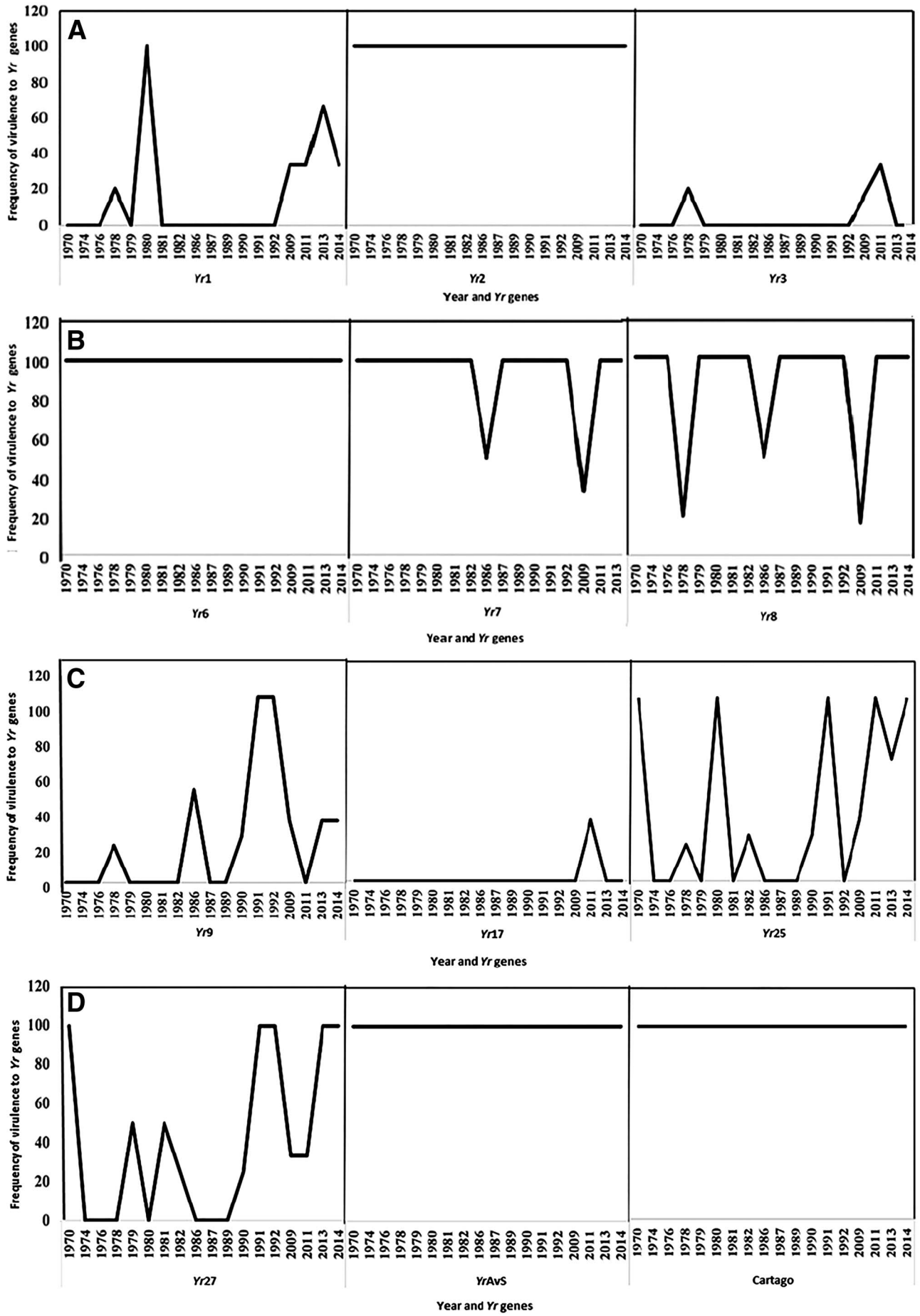


**Fig. 1.** Frequency of virulences for yellow rust (*Yr*) genes seen in *Puccinia striiformis* f. sp. *tritici* isolates collected from across wheat growing regions in Kenya between 1970 and 2014. **A,** *Yr1*, *Yr2*, and *Yr3*; **B,** *Yr6*, *Yr7*, and *Yr8*; **C,** *Yr9*, *Yr17*, and *Yr25*; and **D,** *Yr27*, *YrAvS*; and susceptible control Cartago.

virulences to these genes are fixed in some continents such as Asia, and the latter was introduced in the CIMMYT program as a source of stripe rust resistance (McIntosh et al. 1995). The shift in the prevalent Race 2 (virulence to *Yr2*, *Yr6*, *Yr7*, *Yr8*, and *YrAvS*) to Race 11 (virulence to *Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27*, and *YrAvS*) suggests that *Yr9* and *Yr27* have been deployed in wheat cultivars grown in East Africa, probably as a result of the use of CIMMYT wheat germplasm in wheat breeding programs. Work on gene deployment has been done on wheat stripe rust (Lan et al. 2017). In addition, *Yr* genes have been postulated for various Kenyan wheat genotypes (Wamalwa et al. 2019) and allele diversity of several of *Yr* genes determined in Kenyan and Ethiopian wheat genotypes (Wamalwa et al. 2020).

In this study, virulence to *Yr9* was observed in the Kenyan *P. striiformis* f. sp. *tritici* isolates as early as 1978 (Race 12). Virulence to *Yr9* was first reported in East Africa, in Ethiopia, in 1986 (Singh et al. 2004), but more recent studies of the Stubbs collection have shown that *Yr9*-virulent *PstS1* was present in a Kenyan isolate sampled in 1982 (Walter et al. 2016). Virulences to *Yr9* and *Yr27* are now common and have been reported in Pakistan, Nepal, Turkey, Uzbekistan, the Middle East, South Asia, Canada, and the United States (Bahri et al. 2011; Brar and Kutcher 2016; Sharma-Poudyal et al. 2013; Wan and Chen 2014). Ali et al. (2017) also reported aggressive and temperature adapted lineages *PstS1* and *PstS2* in Asia, the Middle East, and Africa that possess virulence to *Yr27*.

The stripe rust resistance gene *Yr17* is an all-stage resistance gene (McIntosh et al. 1995; Milus et al. 2015). Virulence to *Yr17* was detected in Race 14 in 2011 and has been detected in isolates from Chile, China, and Turkey (Sharma-Poudyal et al. 2013), the United Kingdom and Denmark (Bayles et al. 2000; Hovmøller 2007; Hubbard et al. 2015), the United States (Wan and Chen 2014; Wan et al. 2016), Canada (Brar and Kutcher 2016), and Ethiopia (Wan et al. 2017).

In this study, no virulence was detected for *Yr4*, *Yr5b* (*YrSP*), *Yr10*, *Yr15*, *Yr32*, and *YrAmb*. Similarly, Sharma-Poudyal et al. (2013) did not detect virulence to *YrSP*, *Yr15*, and *Yr32*, but detected virulence to *Yr10* in one of the four isolates from Kenya in 2006. The absence or low frequency of virulence for these resistance genes probably indicates that these *Yr* resistance genes have not been deployed in Kenyan wheat breeding programs or that no virulence has yet evolved in this region.

In Ethiopia, no virulence to *Yr5a* (*Yr5*), *Yr5b*, *Yr15*, and *Yr76* (*YrTye*) was detected in 97 isolates collected in 2013 and 2014

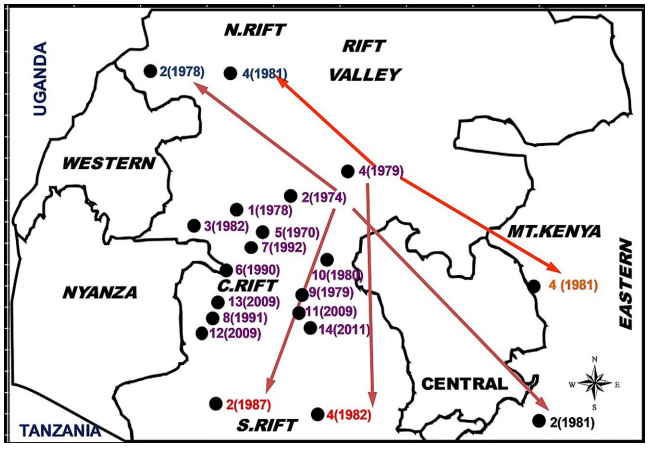


**Fig. 2.** Distribution of virulence to yellow rust (*Yr*) resistance genes present in *Puccinia striiformis* f. sp. *tritici* isolates collected across the wheat growing regions of Kenya between 1970 and 2014. Race 1 (*Yr2*, *Yr6*, *Yr7*, and *YrAvS*), Race 2 (Race 1 + *Yr8*), Race 3 (Race 2 + *Yr25*), Race 4 (Race 2 + *Yr27*), Race 5 (Race 3 + *Yr27*), Race 6 (Race 3 + *Yr9*), Race 7 (Race 4 + *Yr9*), Race 8 (Race 7 + *Yr25*), Race 9 (Race 2 + *Yr1*), Race 10 (Race 9 + *Yr25*), Race 11 (Race 10 + *Yr27*), Race 12 (*Yr1*, *Yr2*, *Yr3*, *Yr6*, *Yr9*, *Yr25*, and *YrAvS*), Race 13 (*Yr2*, *Yr3*, *Yr6*, *Yr25*, and *YrAvS*), and Race 14 (Race 3 +*Yr3* and *Yr17*). Arrows indicate the detection of Races 2 and 4 in different regions after being detected for the first time in the Central Rift Valley.

(Dawit et al. 2012; Wan et al. 2017). Sharma-Poudyal et al. (2013) also observed a lack of virulence to *Yr5a*, *Yr15*, *Yr24* and *Yr32* in an international collection of *P. striiformis* f. sp. *tritici* isolates from Asia and Africa, including Kenya. However, virulence to *Yr10* has been detected in Kenya in 2006 (Sharma-Poudyal et al. 2013) as mentioned previously, also in Eritrea, and at a lesser extent in the Middle East (Ali et al. 2017; Hovmøller et al. 2008, 2016). The differences in virulence found between regions may be due to differences in the *Yr* resistance genes deployed in wheat cultivars grown in those regions or differential responses of the genotypes to environment factors (Danial et al. 1994; Jaetzold et al. 2010; Leonard 2001; Wanyera 1994). Some of the differences also can be due to different wheat genotypes used in different studies to represent specific *Yr* genes. For example, Sharma-Poudyal et al. (2013) used the *Yr10* near-isogenic line in the Avocet S background, and we used Moro, which has *Yr10* and *YrMor* (Chen et al. 1995), in the present study.

As in the present study, regional differences in the presence of *P. striiformis* f. sp. *tritici* races and frequencies of virulences have been observed in Ethiopia. Virulences to stripe rust genes *Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr25*, and *Yr28* were >40% in Arsi Robe (07°2′N and 38°9′E and 2,043 m above sea level) in southeastern Ethiopia but <40% in Holeta (09°0′N and 38°5′S and 2,391 m above sea level) found in central highlands of Ethiopia. These differences were related to differences in cropping systems and the wheat cultivars grown in these two locations (Wan et al. 2017).

The SCAR markers developed by Walter et al. (2016) were used to discriminate the two aggressive high-temperature invasive *P. striiformis* f. sp. *tritici* strains *PstS1* and *PstS2*. East Africa is thought to have been the origin of *PstS1*, being detected in isolates collected in 1982 and 1986. Isolates later spread to the Americas in 2000 and Australia in 2002 (Ali et al. 2017; Brar et al. 2018; Walter et al. 2016), leading to stripe rust epidemics in the United States and Western Australia (Chen 2005; Milus et al. 2009; Wellings 2007). *PstS2* was detected in 2000 in Europe, 2003 in Central Asia, 2005 and 2006 in the Mediterranean area, and 2005 and 2009 in the Middle East (Walter et al. 2016). However, *PstS2* was of lesser economic importance than many wheat cultivars being found to be resistant to *PstS2* (Hovmøller et al. 2016). In North America, Australia, and Europe, *PstS1* and *PstS2* differed from the local, pre-2000 *P. striiformis* f. sp. *tritici* population in aggressiveness and ability to adapt to high temperature (Hovmøller et al. 2011; Milus et al. 2009).

In the present study, SCAR markers SCP19M24 and SCP12M26 were used to screen the Kenyan *P. striiformis* f. sp. *tritici* isolates for the *PstS1* and *PstS2* strains. The old Kenyan isolates from the Stubbs collection were classified as *PstS1* or others, whereas isolates belonging to *PstS2* were only found at one location, Njoro, in 2014. The oldest Kenyan isolate, collected in 1974, was categorized as *PstS1*, indicating that the lineage was present in Kenya before 1980s when *PstS1* was first reported (Walter et al. 2016). Eleven of the 14 races belonged to *PstS1*, indicating that considerable variation in virulence had occurred since the establishment of *PstS1*. The isolates categorized as other strains did not belong to either *PstS1* or *PstS2*. Further work is needed to determine the relationships of these isolates of other strains to *PstS1* and *PstS2*.

In conclusion, comparison of the *P. striiformis* f. sp. *tritici* isolates collected in this study (2009 to 2014) with the old isolates in the Stubbs collection indicated changes in the number of virulences. Eleven races were detected among the isolates from the Stubbs collection, with Race 2 being the most predominant. Among the more recent isolates, four races were found with Race 11 being the most common. Virulences to *Yr2*, *Yr6*, and *YrAvS* were present in all 14 races. Both the old and new isolates collected belonged predominantly to the *PstS1* strain, and only three isolates collected near Njoro in 2014 belonged to *PstS2*. This study therefore supports the conclusions of Walter et al. (2016) that *PstS1* arose in East Africa, although we find evidence of an earlier establishment than previously reported.

The ability of new virulences and races to rapidly emerge within the *PstS1* strain shows the importance of pathogen surveys. By continually surveying and pathotyping more *P. striiformis* f. sp. *tritici*

**Table 4.** Genotyping *Puccinia striiformis* f. sp. *tritici* isolates collected in Kenya from 1970 to 2014 via sequence characterized amplified regions (SCAR) markers

| No. | Virulence profile | Isolate | Year of collection | Location | Region | SCP19M24[a] | | SCP19M26[a] | | *P. striiformis* f. sp. *tritici* strain |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | a1 | a2 | a1 | a2 | |
| 1 | *Yr2*, *Yr6*, *Yr7*, *YrAvS* | KE78105 | 1978 | Ngongongeri | Central Rift | + | + | + | + | *PstS1* |
| | | KE89009 | 1989 | Njoro | Central Rift | n.d. | n.d. | n.d. | n.d. | n.d. |
| 2 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *YrAvS* | KE74195 | 1974 | Molo | Central Rift | + | + | + | + | *PstS1* |
| | | KE76004 | 1976 | Njoro | Central Rift | + | + | + | + | *PstS1* |
| | | KE78019 | 1978 | Njoro | Central Rift | + | + | + | + | *PstS1* |
| | | KE78102 | 1978 | Makutano Nakuru | Central Rift | + | + | + | + | *PstS1* |
| | | KE78107 *Yr8* | 1978 | Eldoret | Northern Rift | + | + | + | + | *PstS1* |
| | | KE81010 | 1981 | Njoro | Central Rift | + | + | + | + | *PstS1* |
| | | KE81046 | 1981 | Subukia | Central Rift | + | + | + | + | *PstS1* |
| | | KE81111 | 1981 | Mununga | Eastern | + | + | + | + | *PstS1* |
| | | KE81082 | 1981 | Kaptagat | Northern Rift | + | + | + | + | *PstS1* |
| | | KE81086 | 1981 | Molo | Central Rift | + | + | + | + | *PstS1* |
| | | KE82017 | 1982 | Mt. Kenya | Mt. Kenya | + | + | + | + | *PstS1* |
| | | KE82044 | 1982 | Endebess | Northern Rift | + | + | + | + | *PstS1* |
| | | KE86063 | 1986 | Njoro | Central Rift | + | + | + | + | *PstS1* |
| | | KE87035 | 1987 | Ngorengore | Southern Rift | + | + | + | + | *PstS1* |
| | | KE87048 | 1987 | Mau Narok | Central Rift | + | + | + | + | *PstS1* |
| | | KE87071 | 1987 | Njoro | Central Rift | + | + | + | + | *PstS1* |
| | | KE89008 | 1989 | Njoro | Central Rift | + | − | + | + | *PstS1* |
| 3 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr25*, *YrAvS* | KE82046 | 1982 | Molo | Central Rift | + | + | + | + | *PstS1* |
| 4 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr27*, *YrAvS* | KE79001 | 1979 | Molo | Central Rift | + | + | + | + | *PstS1* |
| | | KE81070 | 1981 | Maralal | Northern Rift | + | + | + | + | *PstS1* |
| | | KE81040 | 1981 | Mt. Kenya | Mt. Kenya | + | + | + | + | *PstS1* |
| | | KE82026 | 1982 | Marania farm | Mt. Kenya | + | + | + | + | *PstS1* |
| | | KE82031 | 1982 | Maralal | Northern Rift | + | + | + | + | *PstS1* |
| 4 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr27*, *YrAvS* | KE82028 | 1982 | Ngorengore | Southern Rift | + | + | + | + | *PstS1* |
| | | KE82029 | 1982 | Embori | Mt. Kenya | + | + | + | + | *PstS1* |
| | | KE90083 | 1990 | Mau Narok | Central Rift | + | − | + | + | *PstS1* |
| 5 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr25*, *Yr27*, *YrAvS* | KE70063 | 1970 | Molo | Central Rift | − | + | + | − | Others |
| | | KE82043 | 1982 | Eldoret | Northern Rift | + | + | + | + | *PstS1* |
| | | KE328/13 | 2013 | Njoro, KALRO | Central Rift | + | + | + | + | *PstS1* |
| 6 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *YrAvS* | KE90006 | 1990 | Njoro | Central Rift | + | + | + | + | *PstS1* |
| 7 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr27*, *YrAvS* | KE92011 Opata | 1992 | Njoro | Central Rift | + | + | + | + | *PstS1* |
| | | KE92011 VPM1 | 1992 | Njoro | Central Rift | + | + | + | + | *PstS1* |
| 8 | *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27*, *YrAvS* | KE91013 | 1991 | Njoro | Central Rift | + | + | + | + | *PstS1* |
| 9 | *Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *YrAvS* | KE79067 | 1979 | Njoro | Central Rift | − | + | + | + | Others |
| 10 | *Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr25*, *YrAvS* | KE80022 | 1980 | Njoro | Central Rift | + | + | + | + | *PstS1* |
| 11 | *Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27*, *YrAvS* | KE17/09 | 2009 | Njoro, KALRO | Central Rift | n.d. | n.d. | n.d. | n.d. | n.d. |
| | | KE18/09 | 2009 | Njoro, KALRO | Central Rift | n.d. | n.d. | n.d. | n.d. | n.d. |
| | | KE19/09 | 2009 | Njoro, KALRO | Central Rift | n.d. | n.d. | n.d. | n.d. | n.d. |
| | | KE23/09 H.VIII | 2009 | Njoro | Central Rift | − | + | + | + | Others |
| | | KE126a/11 | 2011 | Njoro, KALRO | Central Rift | n.d. | n.d. | n.d. | n.d. | n.d. |
| | | KE132b/11 | 2011 | Mathangauta | Central Rift | + | + | + | + | *PstS1* |
| | | KE327/13 | 2013 | | Central Rift | + | + | + | + | *PstS1* |

(*Continued on next page*)

[a] a1, allele 1; a2, allele 2; n.d., no data; +, presence, −, absence.

**Table 4.** (*Continued from previous page*)

| No. | Virulence profile | Isolate | Year of collection | Location | Region | SCP19M24[a] a1 | SCP19M24[a] a2 | SCP19M26[a] a1 | SCP19M26[a] a2 | *P. striiformis* f. sp. *tritici* strain |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Njoro, KALRO | | | | | | |
| | | KE127/14 | 2014 | Njoro, KALRO | Central Rift | + | + | − | + | *PstS2* |
| | | KE129/14 | 2014 | Njoro, KALRO | Central Rift | + | + | − | + | *PstS2* |
| | | KE131/14 | 2014 | Njoro, KALRO | Central Rift | + | + | − | + | *PstS2* |
| 12 | *Yr1*, *Yr2*, *Yr3*, *Yr6*, *Yr9*, *Yr25*, *YrAvS* | KE78107 TP | 1978 | Eldoret | Northern Rift | − | + | + | + | Others |
| 13 | *Yr2*, *Yr3*, *Yr6*, *Yr25*, *YrAvS* | KE23/09 V23 | 2009 | Njoro | Central Rift | + | + | − | + | *PstS2* |
| 14 | *Yr2*, *Yr3*, *Yr6*, *Yr7*, *Yr8*, *Yr17*, *Yr25*, *YrAvS* | KE138b/11 | 2011 | Mailinne | Central Rift | + | + | + | + | *PstS1* |

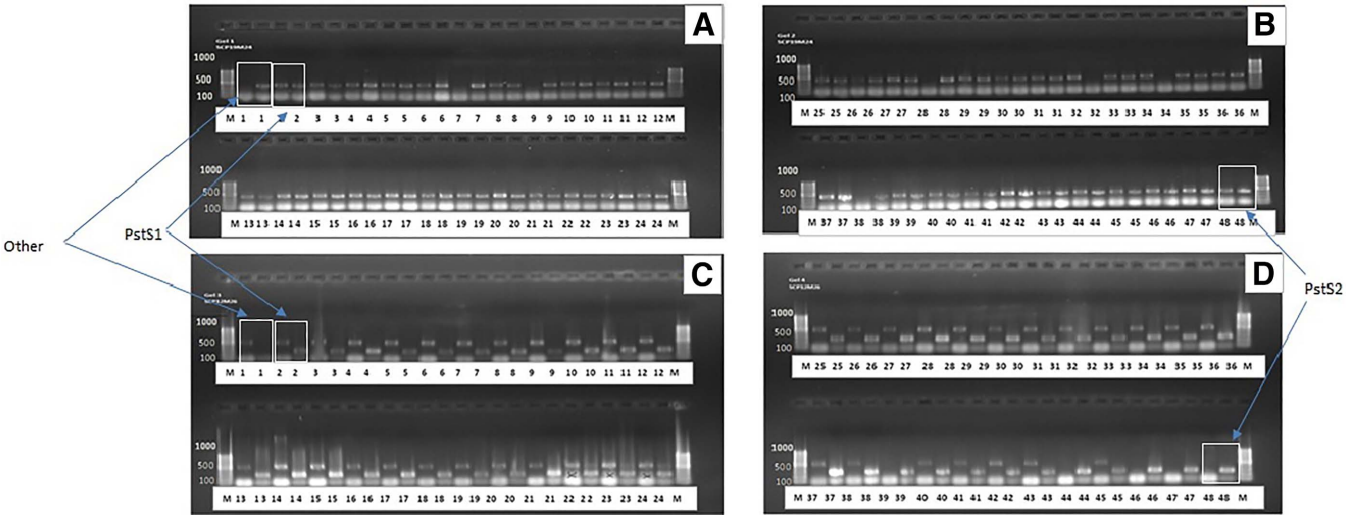


**Fig. 3.** Amplification of genomic DNA from *Puccinia striiformis* f. sp. *tritici* isolates collected from wheat growing regions in Kenya from 1970 to 2014. PCR products were separated on 1.5% agarose gels. The size scale for the DNA bands is shown on the left in base pairs. The primers used are sequence characterized amplified region markers **A, B,** SCP19M24 and **C, D,** SCP12M26. Note: Isolates 29, 45, and 46, whose virulence phenotypes were not assigned, are excluded.

isolates, wheat breeders are informed of the most prevalent virulences and races and know which combinations of stripe rust resistance genes would still be effective. From the work in this study, we selected four races, three pre-2000 (Race 1 with virulences to *Yr2*, *Yr6*, *Yr7*, and *YrAvS*; Race 2 with virulences to *Yr2*, *Yr6*, *Yr7*, *Yr8*, and *YrAvS*; and Race 5 with virulences to *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr25*, *Yr27*, and *YrAvS*) and one post-2000 (Race 11 with virulences to *Yr1*, *Yr2*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr25*, *Yr27*, and *YrAvS*) to be used in screening wheat germplasm for resistance to stripe rust.

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