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Analysis of the population structure 2015 *Puccinia graminis* f. sp *tritici* (Pgt) in Kenya using simple sequence repeats markers

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Abstract

N Wheat (*Triticum astevium*) production in Kenya has been severely affected by stem rust Ug99 and its related race groups. The consequence of not controlling this disease is steep decline in the crop production thus creating food insecurity to over 70% of the small-scale holder farmers who depend on it as a source of food as well as income. The causative agent of the disease *Puccinia graminis* f. sp *tritici* (Pgt) has been studied by researchers globally because of its rapid evolution of races within lineage overcoming existing resistant genes. Understanding the population structure will highlight the predominant race(s) as well as their geographical distribution. This information is required to enable breeding for resistant wheat varieties. The objective of this study was to characterize the population structure of *Puccinia graminis* f. sp *tritici* population in 2015. Using 10pgt Simple Sequence Repeats (SSR) markers 104 single uredenial-pustule samples were analysed. Minimum spanning network pattern was composed of five Simple Sequence Repeats multi-locus genotypes (SSR-MLGs) that were organized around three nodes based on samples chosen from wheat growing fields with the reference isolates; races TTKSK and TKTTF. In addition to this, non-parametric DAPC analysis showed the presence of single population made up of two predominant races from clade I (Ug99 race group) and clade IV-B (race TKTTF/TTTTF). Analysis of molecular variance (AMOVA) according to Bayesian Information Criterion showed clustering was majorly based within populations (0.576%) rather than among clusters (0.441%).

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Introduction

In Kenya, the wheat sub-sector contributes to approximately KSh 5283.1 million annually as per 2017. This was a decelerated rate from Ksh 8198.2 in 2015 to Ksh 5283.1 in 2017 (Kenya Economic Survey 2018). The major constraint of wheat productivity ranged as from biotic and abiotic stresses with emphasis on emerging and re-emerging of stem rust Ug99. This crop employs considerable number of the Kenya population either directly or indirectly with 73% constituting shares of small farms (Kenya Economic Survey 2018). In 2017, 165.2 thousand tonnes was produced a decline of 23.1% from 214.7 thousand tonnes in 2016. To meet her demands for wheat, Kenya currently import 750,000 tonnes annually to fill the deficit consumption of an estimate of one million tonnes (AFA, 2018). The per capita wheat consumption has been increasing by approximately 4% per annum as compared to maize (the main staple cereal) at 1% (AFA, 2018). This is due to rising demand for wheat caused by high population growth, changing eating habits and increased urbanization.

Stem rust caused by *Puccinia graminis* Pers,: Per. f. sp. *tritici* Eriks. and E. Henn (*Pgt)* causes significant losses to wheat (*Triticum aestivum*) production worldwide. It is a pathogen that belongs to rust fungi (Phylum *Basidiomycota*, Order *Pucciniales*) constituting one of the most studied group of fungal plant pathogens (Dean *et al*., 2012). This is one of the most destructive diseases of wheat and barley since it infects spikes, leaf blades, leaf sheath, glumes and stem leading to yield losses up to 100% if not controlled early (Roelfs *et al*., 1992; Park *et al*., 2011). It has been described as an emerging disease due to its increased incidence, geographic and host range, and finally change in pathogenesis because of constant evolution (Hovmøller *et al*., 2011; Singh *et al*., 2011). These coupled characteristics make the pathogen become a threat to global food security and social stability (Vurro *et al*., 2010).

In 1998, stem rust was observed on a breeding line containing the stem rust resistance gene (*Sr31*) in Uganda. This was the first observation that the wheat stem rust pathogen was able to break down this resistance gene. The following year an isolate derived from this breeding line was characterized (*Pgt* race TTKSK) and designated Ug99 (Pretorius *et al*., 2000). In 2004, race TTKSK (Ug99, with virulence to *Sr31*) caused severe epidemic in most wheat growing regions in Kenya (Wanyera *et al*., 2006). Since then, Kenya has witnessed drastic reductions in yield due to emergence of other new combinations of virulence in*pgt* races (Ellis *et al.,* 2014).

Recent studies suggest that 13 variants within Ug99 race group (clade I genotype) have been identified in Africa and parts of Middle East, with 10 of the known variants existing in Kenya (Singh *et al*., 2015; Newcomb *et al*., 2016). Two newly released cultivars 'Digalu' and 'Robin' that were classified with stem rust resistance (*Sr*Tmp) in Ethiopia and Kenya respectively were highly susceptible in 2013/2014 and 2015 in the main wheat growing season.

For Kenya to improve on its wheat breeding program, robust surveillance of Ug99 and its variants needs to be undertake. This is with the aim of knowing the effective and ineffective wheat stem rust resistant genes (*Sr*). Traditionally, this was done through race typing which is time consuming. With the advent of rapid diagnostic tools with emphasis on Simples sequence repeat markers, race typing has been timely and precise.

These findings underscored the importance of understanding Kenya'spgt population structure in major wheat growing regions through genotyping single collected uredinial-pustule samples using tenpgt Simple Sequence Repeats (SSR) markers. The broader objective of this study was to characterize the population structure of *Puccinia graminis* f.sp *tritici* population in the year 2015.

Materials and methods

Study areas

A total of 222 single uredinial-pustules were collected from the wheat farms in the four main wheat growing regions; Mount Kenya (54 samples), Central Rift (77 samples), North Rift (74 samples) and South Rift (17 samples).

DNA extraction method

DNA was extracted using a modification of CTAB method previously described by Doyle and Doyle (1990) with slight modifications of the addition of 20% of Sodium Dodecyl Sulphate (SDS).

Genotyping of Samples

The uredinial-pustules were later genotyped using 10pgt SSR markers: Pge142, Pge227, Pge293, pgtGAA8, pgtCAA53, Pgesstssr 024, pgtCAA80, Pgesstssr 109, pgtCAA49 and Pgesstssr 318 (Szabo, 2007). Products from PCRs were amplified in a MJ Research PTC-200 Gradient Thermal Cycler (Marshall Scientific, Hampton USA) thermocycler using 'Ruth SSR touch' profile.

Data Analysis

The SSR fragment sizing was conducted with Geneious Microsatellite Plugin 1.4 for Geneious R7 software. SSR data was analysed using R (version 3.1.2) software 'Poppr' package with additional libraries of Ape v3.1-4 (Paradis *et al*., 2004), Adegenet v 1.4-2 (Jombart 2008) and Pegas v 0.6 (Paradis *et al*., 2004) used for phylogenetic analysis. The function 'aboot' was used for constructing trees with emphasis on the Nei's distance (Nei, 1978), neighbor-joining (Saitou and Nei, 1987), a sample of 5,000 bootstrap replicates and a cut off of 5%. GenoDive (K-Means Clustering: Meirmans and Van Tienderen, 2004) software was used to generate Analysis of Molecular Variance (AMOVA) data based on Bayesian Information Criterion.

Results

A total of 104 single uredinial-pustule collections were successfully genotyped out of the total collected 222 samples. Five SSR-MLGS were amplified. Mount Kenya had the highest number of SSR Multi-locus genotype (4 SSR-MLGs) while South Rift had the least (2 SSR-MLGs). Central Rift and North Rift had equal distribution of (3 SSR-MLGs) each. SSR-MLG.02 was observed across the four regions analysed (Mount Kenya, Central Rift, North Rift and South Rift) with the highest frequency being in Central Kenya (55%) and least frequency in Mount Kenya (10%). Based on SSR-MLG genotypes of reference isolates, clade IV-B (race TKTTF/TTTTF) was associated with SSR-MLG.03 while clade I (Ug99 race group: TTKSK) was represented with SSR-MLG.01. (Fig 1.)

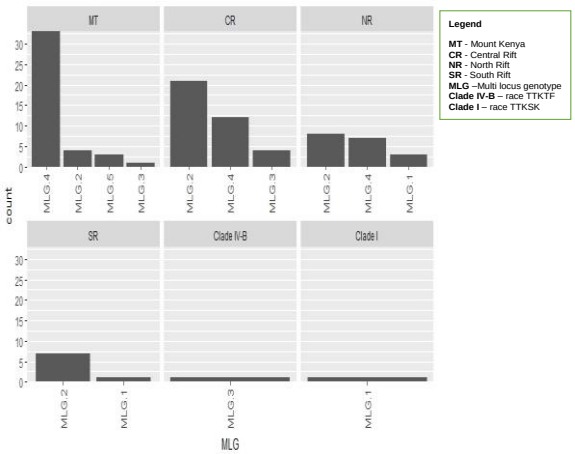

Fig 1. Simple Sequence Repeats-Multiple locus genotype (SSR-MLGs) for 104 Kenyan *Puccinia graminis* f. sp. *tritici* samples of 2015 based on 10pgt single sequence repeats loci used for the analysis.

To understand the genetic relation between five SSR-MLGs, the data set containing 104 samples and two referencepgt isolates clade I (race TTKSK) and clade IV-B (race TKTTF), minimum spanning network was drawn which organized three nodes of SSR-MLG.04, SSR-MLG.03 and SSR-MLG.05. SSR-MLG.01 was associated with clade I (Ug99 race group: TTKSK) majorly found in South Rift and North Rift while SSR-MLG.03 was associated with clade IV-B (race TTKTF/TTTTF) found in Central Rift and Mount Kenya regions. From the network pattern, SSR-MLG.05 was identified as unique SSR-MLG with Mount Kenya having the highest number. Both SSR-MLG.02 and SSR-MLG.01 sub-networked with SSR-MLG.05. Central Rift had the highest number of SSR-MLG.03, SSR-MLG.01 highest in North Rift and SSR-MLG.04 highest in Mount Kenya (Fig. 2.).

The data was analyzed using multivariate method: non-parametric Discriminant Analysis of Principle Components (DAPC) in order to identify and describe clusters of genetically related individuals from the sampled regions. The results showed no evidence of sub-structure based on geographical locations sampled (Fig. 3.).

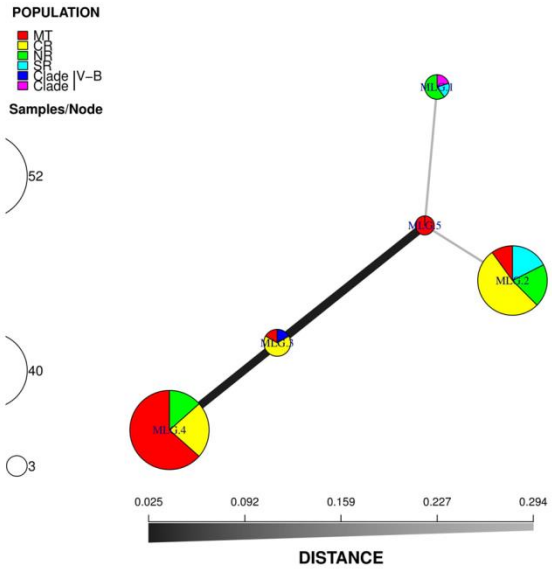

Fig. 2. Minimal spanning network (MSN) of 104 Kenyan *Puccinia graminis* f.sp. *tritici* samples of 2015. In addition, reference isolates clade I (race TTKSK) and clade IV-B (race TKTTF) were included in the analysis.

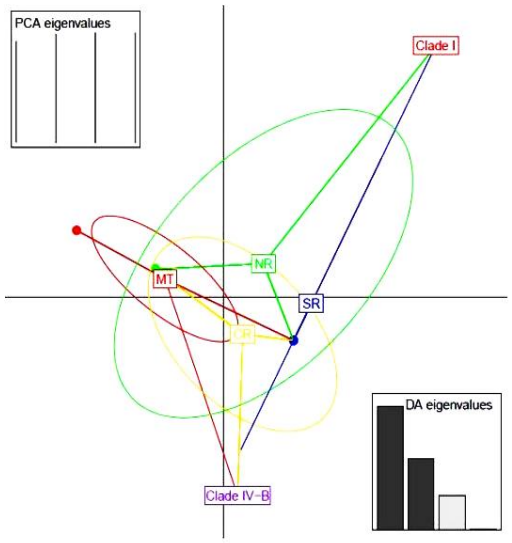

Fig. 3. Scatterplot from discriminate analysis of principle components (DAPC) of the first two components discriminating of pgt fields samples by genetic groups based on region to region level (Mount Kenya (MT), Central Rift (CR), North Rift (NR) and South Rift (SR)).

AMOVA according to Bayesian Information Criterion showed clustering was majorly based within populations (0.576%) rather than among clusters (0.441%). (Table 1)

Table 1. Analysis of Molecular Variance on best clustering according to Bayesian Information Criterion (BIC).

Source of Variation	Nested In	SSD	d.f	MS	%var	Rho	Rho-value
Within Populations	--	260.009	100	2.600	0.576	st	0.424
Among Populations	Clusters	5.631	3	1.877	-0.017	sc	-0.031
Among Clusters	--	121.049	2	60.524	0.441	ct	0.441
Total (SST)	--	386.689	105	3.683	--	--	

SSD: Sum of Squares; d.f: degree of freedom; MS: Mean Squares; Rho: An F*st* analogue that is independent of the ploidy level

Discussions

This data was to supplement the study conducted in 2011 with the main agenda of characterizing stem rust population in Kenya through the utilization of simple sequence repeats markers (SSR) (Wanyera *et al.*, 2017 in press). This is because of specialization of the pgt races which has strongly impacted on wheat breeding and production considering that 10 known variants of Ug99 race groups have been observed in Kenya from the total 13 globally described variants (Newcomb *et al.*, 2016).

The most interesting results of this study was the introduction of clade IV-B (race TKTTF) into Kenya pgt population which was initially observed in Ethiopia and responsible for breaking down Digalu variety (Olivera *et al.*, 2015). This would confirm that the pgt spores is dispersed via airborne based on previous studies of the utilization of U.K Met Office Numerical Atmospheric-dispersion Modeling Environment (NAME) model (Jones *et al.*, 2007).

The equal distribution of the five multi-locus genotypes in the major wheat growing regions suggested low level of genetic diversity. Despite there being long-distance dispersal and spread of new clade IV-B (race TKTTF) from Ethiopia, there was no clear genetic separation structure in the sampled regions. This was not consistent with the previous studies done on *Puccinia striformis* f.sp *tritici* (PST) in Asia whereby the local population had high level of genetic diversity after the invasion of new PST strains (Singh *et al.*, 2004; Brown *et al.*, 2002). In addition to this, it would be noted that the 2015pgt population was shaped by the continual invasion of new aggressive

races which have virulence to the already deployed *Sr* (resistant) genes. In this case, the widely grown wheat variety Robin that had *Sr*Tmp gene contributed to the replacement of the pgt population with the introduction as clade IV-B (race TKTTF) which was virulent to that gene. This distribution would be supported by the step wise range expansion as the wheat growing regions are close to one another (Singh *et al.,* 2004). Furthermore, increase of prevalent abiotic stress in this case high temperatures in range temp of 16^{o}C - 27^{o}C greatly determined the outcome of introduction of new race TKTTF from Ethiopia into the local Kenya population. This is considered as the ideal environment for the pathogen formation (Roelfs, 1992). This is further supported by networking five multi-locus genotypes in the minimum spanning network with major reference isolates from clade I (race TTKSK) and clade IV-B (race TKTTF). The fact that the DAPC analysis showed no evidence for sub-structure based on geographical location and the gradual decay in eigenvalues rather than steep observed as the AMOVA supported the clustering to be within population (0.576%) rather than among clusters (0.441%). Similar results were obtained in 2011pgt population studies (Wanyera *et al.*, 2017 in press).

Conclusion

The 2015pgt population in Kenya is made up of two general SSR genotypes group: clade I (Ug99 race group, TTKSK) and clade IV-B (TKTTF). This is a shift from the previous 2011 Kenya pgt population which was only made up of clade I (Ugg 99 race group). In addition to this, the pgt population is single based on non-parametric DAPC and AMOVA analysis. With these findings, Kenya wheat breeders will develop wheat cultivars having multiple effective stem rust resistant genes in relation to the known races in Kenya and surrounding regions. Once these resistant breed varieties are available to wheat farmers, it is expected to help them improve in their yield and maximize on their return through sales.

Recommendations

Implementing diagnostic tools for rapid detection of stem rust races at KALRO, Kenya has proved to be reliable and robust. In return, wheat breeders are encouraged to understanding pathogenic diversity thus deploying resistant effective genes against various races with emphasis on Clade I (Ug99 race group) and Clade IV-B (Digalu race groups)

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Declaration of interests

The authors declares that they have no conflict of interest.

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