International Journal of Enology and Viticulture ISSN: 2756-3685 Vol. 11 (5), pp. 001-012, May, 2024. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Outbreak of grapevine leaf roll disease in East Africa: A case study of Tanzania

Herieth Rhodes Mero

University of Dar es Salaam, Mkwawa University College of Education (MUCE), Faculty of Science, Department of Biological Sciences, P. O. BOX 2513, Iringa, Tanzania.

Email addresses of the Corresponding author

heriethmero2@gmail.com; herieth.mero@muce.ac.tz; mero.herieth@udsm.ac.tz

ORCID of the Corresponding author

0000-0001-6790-6390

Phone contact of the corresponding author

Mobile phone number: +255 759247662.

Receive April 3, 2024; Accepted May 21, 2024 and Published 6 June, 2024

Abstract

A case-control study was conducted in Dodoma, Tanzania to determine unknown genetic diversity of causal viruses of the most destructive Leaf Roll Disease of Grapevines (GLD) emerged in Chisichili (January 2024) and Mpunguzi (October 2023) villages. Sum of 600 leaf samples from apparently health (controls) and symptomatic GLD-cases of Koja variety combined, were collected from the two villages. By Reverse Transcription Polymerase Chain Reaction assay, type 3 and (3 and 1) of *Grapevine Leaf Roll associated Virus (GLRaV)* species were detected in the corresponding Chisichili and Mpunguzi villages, with prevalence of 54 (54%) and (164(82%) and 12(6%)), respectively. Forty-six (46%) and 24 (12%) of the suspected GLD-cases in the respective Chisichili and Mpunguzi villages, tested negative for the investigated 1, 2, 3, 4 and 7 types of the GLRaV species including all (300(100%)) control samples. Identification of the GLRaV-3 and 1 in Tanzania calls for immediate improvement of grapevine phytosanitation practices and implementation of strict quarantine policy to prevent spread of the viruses in East Africa. Metatranscriptomic and transcriptomic studies are needed in order to characterize the GLRaV-variants and determine level of virus-resistance in Tanzanian grapevines, correspondingly. Such knowledge is prerequisite to transgenesis and/or breeding of virus-resistant grapevines for effective disease control.

Keywords: *Vitis vinifera*; *Grapevine leaf roll associated virus* species; Grapevine Leafroll Disease; Genetic Diversity; Prevalence; Virulence; Pathogenicity; Variants; Host-pathogen interaction; RT-PCR assay.

INTRODUCTION

Grape is a fruit crop of socio-economic importance produced in the four (Asia, Europe, America and Africa) continents in the world. The ten leading producer countries of the crop in the world are China, France, United States of America (USA), South Africa, Italy, Chile, Iran, Turkey, Spain and Argentina (Khan *et al.*, 2020). Seventy-one percent (71%), 27% and 2% of the produced grapes are consumed in form of wine, fresh fruits and raisins, respectively (Khan *et al.*, 2020). Grapes' benefits are not limited to medicine (Khan *et al.*, 2020) but extended to pharmaceutical and cosmetics industrial applications (Sharafan *et al.*, 2023; Serra *et al.*, 2023).

Viral diseases (Alabi *et al.*, 2016; Song *et al.*, 2021) causes more yield losses in grapevines than the fungal (Csikós *et al.,* 2020) and bacterial (Kyrkou *et al.,* 2018)

diseases. On a global scale grapevine are prone to infection by over 80 virus species, some of them are of economic importance whilst others are not (Xiao *et al.*, 2018). Red blotch, rugose woody complex, infectious degeneration and decline fleck complex are major economic diseases of grapevines in the world but the complex, vector-borne Leaf Roll disease of grapevine (GLD) is the most destructive one (Maree *et al.*, 2013; Xiao *et al.*, 2018). Yield loss contributed by the GLD was reported to be over 40% per vineyard and its economic impact is estimated to be US$25,000 - US$40,000 per hectare for vineyards with 25-years lifespan in Finger Lake grapevines of New York (Atallah *et al.*, 2012). The GLD complex is caused by at least eight (1, 2, 3, 4, 5, 7, 9 and 13) types of the *Grapevine leafroll-associated virus* (GLRaV) species classified into three distinct genera of the *Closteroviridae* family (Maree *et al.*, 2013; Song *et al.*, 2021). Type (1, 3, 4, 5, 6, 7, 9 and 13), 7 and 2 of the GLRaV species belong to *Ampelovirus,Velarivirus* and *Closterovirus* genus, respectively (Almeida *et al.*, 2013; Song *et al.*, 2021). Even though grape is produced in twelve (South Africa (Ras & Vermeulen, 2009; Maponya & Oluwatayo, 2020), Tanzania (Kulwijila *et al.*, 2018), Egypt (El-Saeed *et al.,* 2015), Ethiopia (Berhe & Belew, 2022),Tunisia (Mansour *et al.*, 2011), Madagascar (Kok, 2014), Libya Abousef, 2020), Namibia (Eden *et al.*, 2020), Zimbabwe (Phiri *et al.*, 2020), Malawi (Khan *et al.*, 2020), Algeria (Laiadi *et al.*, 2009; Rahali *et al.*, 2019) and Morocco (Zinelabidine *et al.*, 2014) countries, the GLD was confirmed to be prevalent in the northern and southern zones of the African continent only. The GLRaV-1, (1, 2 and 3) and (2, 3, 5 and 6) were characterized in South Africa (Coetzee *et al.*, 2010), Egypt (Ahmed *et al.*, 2004, 2007) and Algeria (Lehad *et al*., 2015), respectively.

Unfortunately, prevalence of the GLD and genetic diversity of its causal GLRaV species is unknown in the eastern African producer countries (Ethiopia and Tanzania).

The present study focused on investigation of the GLD in Tanzania, the second largest grapevine and wine producer in the African continent after South Africa (Ngaruko, 2022). Currently, grape production in Tanzania has been extended to Morogoro, Kilimanjaro, Tanga, Tabora, Manyara (Babati), Mara (Bunda) and Ruvuma (Peramiho) regions (Ngaruko, 2022), after being cultivated in the Dodoma region for over five decades and farmers rely on the crop as a source of foreign currency and employment (Kulwijila *et al.*, 2018). In the 2016 and 2017 years, Tanzania produced 4990 and 5192 tons of grapes, correspondingly (Khan *et al.*, 2020). In the 2018/2019 year, 71.57% of grapes produced in Tanzania were traded to both foreign and domestic markets and the government expects to produce 22,000 tons during the 2024/2025 year (Ngaruko, 2022). Nonetheless, outbreak of grapevine diseases (Kulwijila *et al.*, 2018) constrain production of the crop and is one middle block towards achievement of such goal.

Recently, the unprecedented cases of red wine grapevines with evident symptoms akin the complex GLD, were observed in two vineyards located in the Dodoma municipality.

The present molecular investigation was conducted to detect and characterize types of the GLRaV species prevalent in villages with suspected disease outbreak in Dodoma region.

Detection and characterization of the genetically distinct GLRaV species circulating in Tanzania will have a huge implication on the diagnosis, management and control of the disease for alleviation of food insecurity, malnutrition and poverty ascribed to the GLD. Firstly, understanding the genetic structure of the GLRaV species will guide custom design of user friendly, highly sensitive and specific molecular assays for quick routine diagnosing the GLD. By doing so, correct identification of the GLRaV-strains circulating in the country will guide production of virus-resistant grapevines through breeding and/or transgenesis by Ribonucleic Acid (RNA) interference technology. Secondly, confirmation of the GLD prevalence will improve current farming and management practices (phytosanitation and quarantine measures) of grapevines in order to reduce transmission of the GLRaV species through conduction of farmers' awareness campaigns on the disease epidemiology.

MATERIALS AND METHODS

1.1. Study Area

This study was conducted in Chisichili and Mpunguzi villages located in Dodoma, Tanzania where outbreaks of the grapevine leafroll disease were observed in the country for the first time. Mpunguzi is among the four leading producer villages of grapevines in Dodoma (Nyagango *et al.*, 2023) where the crop was first imported and domesticated by the Christian Missionaries in 1930s and hence serve as a potential study area for investigation of introduced grapevine disease and pests. On the other hand, Chisichili village with low grape production is geographically located near the Mpunguzi village and serve as the immediate recipient of planting materials from Mpunguzi and hence could provide information on the patterns of the disease spread across villages in Dodoma region.

The GPS coordinates (latitude and longitude) of the surveyed N1, N2, N3 and N4 farms were (-6.27237 and 35.71957), (-6.39074 and 35.7516), (-6.38989 and 35.75044) and (-6.370667 and 35.753515), respectively.

1.2. Study Design

The current study with a case-control design, was condu-

cted in Mpunguzi and Chisichili villages in October 2023 and January 2024, respectively.

1.3. Sample Collection

By a non-randomized, purposive sampling technique (Campbell *et al.*, 2020), leaf samples were collected from asymptomatic (apparently healthy) red grapevines (Figure 1) and symptomatic cases of the GLD with evident red discoloration on the leaf's lamina, greenish leaf veins and down-ward curling of the leaf from its margin (Kok, 2014; Khan *et al.*, 2020). Symptoms of the GLD observed in red grapevines (Koja variety) are shown in Figure 2. The red grapevines (Koja variety) from which samples were collected, had a 3-5 years of age.
A total of 100 and 200 leaf samples with evident symptoms for the GLD were collected from the Chisichili and Mpunguzi Villages. For each village, an equal number of leaf samples were collected from asymptomatic red grapevines. To maximize detection of viruses, three leaves were collected per plant whereby the first, second and third leaf was sampled at the top (apex), middle and bottom of the stem, respectively.

1.4. Sample Analysis

Total RNA molecules were isolated from grapevine leaves by Cetyltrimethylammonium bromide (CTAB) extraction protocol (Gambino *et al.*, 2008). Purity and concentration of the total RNA extracts were measured by using NanoDrop 2000 Spectrophotometer. Molecular detection and characterization of type 1, 2, 3, 4 and 7 of the grapevine leaf roll-associated virus (GLRaV) species was done by a One Taq One-Step RT-PCR Kit (E5315) from New England Biolabs Inc.A 50µl master mix was prepared by mixing 25 µl of One Taq One-Step Reaction mix (2X), 2 µl of One Taq One-Step Enzyme Mix (25X), 2 µl of gene-specific forward primer (10 µM), 2µlof gene-specific reverse primer (10 µM), 18µlof nuclease-free water and 1 µlof total RNA extract. The forward and reverse primers targeting corresponding HSP70h (379 bp), CP (525bp), CP (301bp), P23/ORF6 (490 bp) and CP (550bp) of the GLRaV-1, 2, 3, 4 and 7 were (5'-TTGAGRGCTCTBATAAAYGAAC-3' and 5'-CGTTMARTTCGYCKACSGACA-3'), (5'-RCDATGGAGYTRATGTCYGA-3' and 5'-AGCGTACATRCTYGCRAACA-3'), (5'-TCTTAAARTAYGTTAAGGACGG-3' and 5'-GGCTCGTTAATAACTTTCGG-3'), (5'-GGACAATTTAGGTAATGTWGTRGCTAC-3' and 5'-TATCCTCAGWGAGGAARCGG-3'), (5'-CTAGTGAATTACACCGAGAAGTC-3' and 5'-GTGACTTGGCACGCATGTATC-3'), respectively (Xiao *et al.*, 2018). One cycle of reverse transcription, 1 cycle of initial denaturation, 40 cycles of (denaturation, annealing and extension),1 cycle of final extension and 1 cycle of hold steps of the RT-PCR reaction were run at respective 48°C, 94°C, (94°C, 50-55°C depending on the used set of virus-specific primers as described in Xiao et al. (2018) and 68°C), 68°C and 4°C for corresponding 15 minutes, 1 minute, (15 seconds, 30 seconds and 1 minute per kilobase pair), 5 minutes and infinity. The PCR amplicons were resolved by electrophoresis using 1% agarose gel at 80 volts and visualized in a LUMINAX gel documentation system. The CSL-MDNA-1Kb DNA Ladder RTU (www.cleverscientific.com) was used as a marker during agarose gel electrophoresis.

1.5. Data Analysis

By using the statistical package, R version 4.3.2 (https://www.r-project.org/, visited on 10th March 2024), prevalence of each of the identified GLRaV species were computed as a proportion of samples which tested positive for a specific virus species, to all virus-tested samples (Sharma *et al.,* 2015). A percentage of virus free samples was calculated as a proportion of samples which tested negative for all investigated GLRaV species to all samples analyzed by the RT-PCR assay.

RESULTS

Purity expressed in terms of absorbance ratio (A_{280}/A_{260}) and concentration of the total RNA extracts had a range of (2.0-2.8) and (8.89 - 17.88 ng/µl), correspondingly.
The present study report presence of the GLRaV-3 and 1 species in the investigated Tanzanian grapevine farms located in Chisichili and Mpunguzi villages respectively (Figure 3). Prevalence of type 3 and (3 and 1) of the GLRaV species in the Chisichili and Mpunguzi villages were 54 (54%) and (164(82%) and 12(6%)), respectively. Forty-six (46%) and 24 (12%) of the suspected cases of the GLD in respective Chisichili and Mpunguzi villages, tested negative for the investigated viruses (types 1, 2, 3, 4 and 7 of the GLRaV species) including all (300(100%)) asymptomatic samples collected from the two villages.

DISCUSSION

This report confirms that the GLRaV-1 and 3 were the causal agents of the GLD outbreaks which occurred in the Chisichili (January 2024) and Mpunguzi (October 2023) villages located in Dodoma, Tanzania**.** With reference to the work by Song *et al.* (2021), the identified GLRaV-3 and 1 are the most prevalent causal pathogens of the GLD in the world and they induce strong disease symptoms in hosts. Based on another study by Maree *et al.* (2013) the former is the most aggressive and widely distributed in the world unlike the latter. Emergence of the debilitating GLD in Tanzania, the major producer of grapes and wine grapes in the East Africa besides Ethiopia (Berhe & Belew, 2022), has huge implications on the crop production and economy in the zone.

Figure 1: Apparently healthy grapevines (Negative controls) with no evident symptoms of the GLD.

One of the challenges encountered by Tanzanian farmers is that the produced grapes are rejected by mega purchasers (wine industries), because they tend to have low sugar concentration below the required standard 23-28°Brix range (Kelly *et al.*, 2018). Sugar composition and their respective quantity are the main factors which determine the quality of berries, alcohol content of the produced wines and hence its marketability (Jordão *et al.*, 2015; Zhong *et al.*, 2023). Sucrose, fructose and glucose are the major carbohydrate compounds constituting the grape berries in addition to antioxidants, organic acids and phenolic compounds (Trad *et al.*, 2017; Lodaya & Gotmare, 2018). However, concentration of these sugars varies inherently between grapevine cultivars and geogra-

A1

A2

B

C

phical zones. For instance, fructose and glucose were the main carbohydrate compounds constituting 17 wild grapes *(Vitis vinifera subsps. Sylvestris)* growing under Mediterranean conditions of Tunisia and their glucose/fructose ratio varied significantly ($p < 0.01$) over a wide range (0.26 - 12.38) (Trad *et al.*, 2017). Contrarywise, 18 cultivars (Summer Black, Bronx seedless, Crimson seedless, Flame seedless, Rizamat, XinYu, Wuhecuibao, Shine Muscat, Victoria, Black Monukka, RedGlobe, Thompson seedless, Centennial

Figure 2: Suspected cases of red grapevines with symptoms of the GLD in Mpunguzi (A) and Chisilichi (B, C, D and E) villages. The GLD symptoms include reddish discolouration on leaf lamina and green veins, downward curling of leaves on the leaf margins (red arrows).

seedless, Munake, Yatomi Rosa, Huozhouheiyu, Huozhouhongyu and Melissa) of grapevines cultivated in Xinjiang, China, were composed of glucose, D-fructose and sucrose with respective 42.13-46.80%, 42.68-50.95% and 6.17-12.69% quantities (Zhong *et al.*, 2023). In the context of this work, information is lackingon the types and quantities of sugars expressed by different grapevine varieties cultivated in Tanzania.

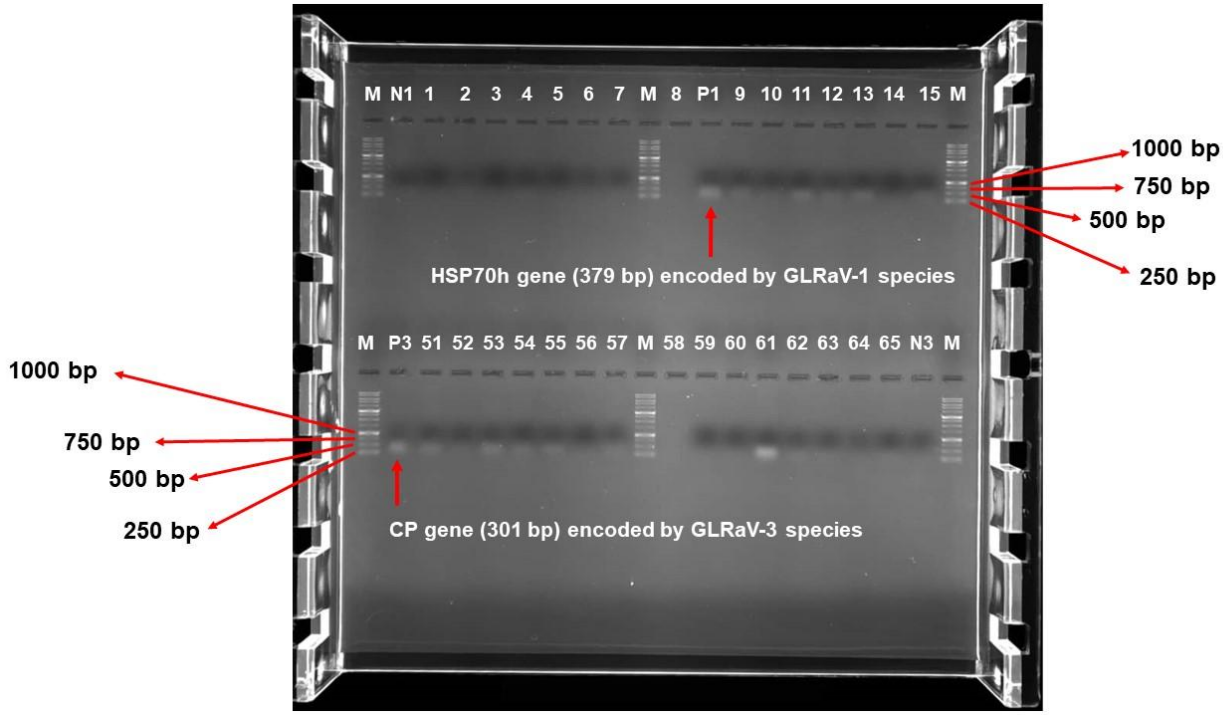

Figure 3: A gel picture showing the GLRaV-1 and -3 species detected by the one-step RT-PCR assay in the Chisichili and Mpunguzi villages. The (N1 and N3) and (P1 and P3) represent (placebos for the GLRaV-1 and 3, separately) and (positive-controls for the GLRaV-1 and 3, separately), respectively.

Identification of grapevine varieties with best sugar composition and content using metabolomics tools (Kumar *et al.*, 2017; Zhong *et al.*, 2023) is essential for improving production of the crop through breeding of the involved genetic resources. Both environmental factors and viticulture management practices influence composition and concentration of sugar in berries (Jordão *et al.*, 2015). Selection of the grape cultivar, geographical location of the vineyard, rootstock and irrigation strategies are some of the viticulture management factors which predetermine concentration of grape sugars during harvest time (Olego *et al.*, 2016). Biological stressors such as occurrence of diseases, composition of soil nutrients and climate change (Olego*et al.*, 2016) are some of the associated environmental factors. High temperature during drought increases sugar composition of grapes (Lakatos & Mitre, 2023) whilst viral diseases like the GLD reduce yield and sugar content (°brix) of grape berries (Alabi *et al.*, 2016) by delaying ripening of the fruits, reducing total soluble solids (TSS) and increasing titratable acidity in fruit juice (Atallah *et al.*, 2012). Additionally, wines from GLD-affected grapevines had significantly lower alcohol, polymeric pigments and anthocyanins compared to asymptomatic grapevines (Alabi *et al.*, 2016). There are no scientific reports which have highlighted the effects of GLD-infection on reduction of sugar content of grape berries and low alcohol content of the produced wines in Tanzania. Majority of Tanzanian grapevine farmers link the problem of sub-standard sugar concentration of grape berries with soil infertility only, because they are unaware of the GLD epidemiology and its economic consequences. These facts necessitate awareness campaigns to all farmers and other stakeholders in the grape wine and juice production chain.

The present case-control study has reported the GLRaV-1 and 3 species prevailing in only two grape producer villages (Mpunguzi and Chisichili) in Dodoma but the conventional RT-PCR assay couldn't provide information on circulating variants/strains of the respective species, contrary to the high-throughput next generation RNA sequencing technologies with high reproducibility, large dynamic range, requirement of less RNA sample and the ability to detect novel transcripts and alternative splicing, even in the absence of a sequenced genome (Costa et al. 2013). Metatranscriptomics is a powerful tool which is highly applied in determination of genetic diversity of both previously known and novel viruses infecting plants. For example, the *(Bean Common Mosaic Virus (BCMV)*, *Bean Common Mosaic Necrosis Virus (BCMNV)* and *Clover*

Yellow Vein Virus (ClYVV)), (Squash vein yellowing virus (SqVYV), Citrus-associated rhabdovirus (CiaRV), and a novel polerovirus-related strain termed *Bitter apple aphid-borne yellows virus (BaABYV))* and *(apple stem grooving virus* and *(citrus leaf blotch virus, cucumber mosaic virus,* and *lychnis mottle virus))* were identified by metatranscriptomics in corresponding common beans (*Phaseolus vulgaris L*.), bitter apple plants (*Citrullus colocynthis* (L.) Schrad) and (pear and kiwifruit) in India (Rashid *et al.* 2022), southern part of Kerman province, Iran (Ghorani *et al.*, 2024) and Korea (Lee & Jeong, 2022), respectively. Therefore, conduction of metatranscriptomic surveys is recommended in order to understand genetic structure of the GLRaV population at sub-species (strain) level, including identification of novel strains of the GLRaV species in the scrutinized and unscrutinized grapevine producer villages (Mvumi, Hombolo, Chinangali, Mpunguzi, Mbabala A, Mbabala B, Mchemwa, Matumbulu and Veyula) (Nyagango *et al.*, 2023) in Dodoma and other grapevine producer regions (Morogoro, Kilimanjaro, Tanga, Tabora, Manyara, Mara and Ruvuma) in Tanzania.Talking of genomic structure of the GLRaV-3 species alone, the virus consists of over 40 variants classified into eight basic phylogenetic groups (I, II, III, V, VI, VII, IX and X) and the species is believed to continue evolving (Bester *et al.*, 2012; Hančević *et al.*, 2023). Variants of the pathogenic viruses varies significantly in terms of their biological properties (transmissibility between hosts and replication rate in hosts) reflected by their genomic variation (Hančević *et al.*, 2023) and that has a big impact on severity of disease symptoms in the plant host and magnitude of economic loss.

Understanding the genomic structure of the variant population of GLRaV species is an important prerequisite to custom design of a more sensitive (quantitative RT-PCR) (Bester *et al.*, 2012) and/or user- friendly (Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) (Deshields & Achala N., 2023) molecular assays for quick, routine diagnosis of the GLD. Additionally, conduction of a country-wide metatranscriptomic survey of the GLD is essential for tracking patterns of disease spread as farmers have a tradition of exchanging grapevine germplasms within and across the regional borders without taking any phytosanitary measures. Since Dodoma used to be the sole grapevine producer region in the country for over five decades, it is most likely that, grapevine genotypes which are recently cultivated in other regions (Morogoro, Kilimanjaro, Tanga, Tabora, Manyara, Mara and Ruvuma) harbour the GLRaV species because most of the planting materials were sourced from Dodoma. The present study speculates that Mpunguzi village is one of the primary sources of the GLRaV species in the country because it is amongst the first villages where the crop (believed to have harboured the viruses), was first introduced from Italy during the colonial era. Another possible source for emergence of the GLD in Tanzania could be from other grapevine producer countries in Sub-Saharan Africa. For instance, 13 elite genotypes of grapevines were imported from South Africa by the Tanzania Agricultural Research Institute (TARI) as per the report released on 29th June 2020 (https://www.wineland.co.za/tanzania-to-introduce-new-grape-varieties-to-boost-wine-production/ visited on 1st April 2024). The GLD is endemic in South Africa (Bester *et al.*, 2012) and there's no record showing where the imported (elite) genotypes of grapevines were disseminated to and cultivated in Tanzania. Furthermore, there's no molecular study which has traced the spread of the GLRaV species from South Africa to Tanzania.

The molecular biology of GLRaV strains circulating in Tanzania is unknown, especially their pathogenicity and virulence levels which determine the magnitude of the economic impact of the GLD to farmers. According to Sacristán & García-Arenal. (2008) virulence and pathogenicity are defined as (quantitative degree of damage caused to a host by parasite infection, assumed to be negatively correlated with host's fitness) and (qualitative capacity of a parasite to infect and cause disease in a host), correspondingly. Understanding the virulence evolution of the GLRaV species is extremely important in the proper planning of the GLD management and control, because it may determine emergence and re-emergence of the disease outbreaks, host switch and host range expansion, and disease resistance-breakdown (Sacristán & García-Arenal, 2008) in improved grapevine genotypes. Moreover, virulence is a measure of virus fitness and determine the magnitude of epigenetic and transcriptomic plants' response during infection (Corrêa *et al.*, 2020).

Transcriptomics (Zanardo et al., 2019; Tyagi et al., 2022) and proteomics (Lodha *et al.*, 2013; Mann & Brasier, 2021) are the two gene expression studies which can be used to characterize the virulence and pathogenicity determinant genes actively expressed by the GLRaV species.

Additionally, there's knowledge scarcity on the host-pathogen interaction paradigm of the GLD in endemic settings of Tanzania. Research on plant-pathogen interactions can lead to identification of genes involved in pathogen tolerance or resistance, or the identification of triggers of plants' defense response (Bester *et al.*, 2017). Talking of virus-and-virus interaction facet, no mixed infection was observed between the detected GLRaV species despite such cases being reported elsewhere. For example, different combinations of mixed infections were observed between (1 and 3), (3 and 4) and (4 and 7) types of the GLRaV species in Portugal (Moutinho-Pereira *et al.*, 2012), Australia (Salo *et al.*, 2024) and Switzerland (Reynard *et al.*, 2015), respectively. Currently, it is unknown whether single or multiple variants of the detected GLRaV-1 and 3 species were responsible for causing the GLD outbreak in the investigated

villages. The GLD cases caused by multiple variants of the same species are not uncommon. For example, multiple variants of the GLRaV-3 species were confirmed to infect four grapevine varieties (Cabernet Franc, Merlot, Pinot Noir and Tribidrag) (Hančević *et al.*, 2023). Understanding the mechanisms underlying mixed infection among the GLRaV species at species and intra-species level has an implication on disease symptomology which is directly associated with magnitude of its economic loss. The severity of disease symptoms depends on whether the GLRaV species or respective variants implicated in the mixed infection have neutral, synergic or antagonistic interactions (Syller & Grupa, 2016).

In the context of plant-virus interaction, baseline knowledge on resistance/tolerance levels of the Tanzanian germplasms against the GLRaV species is currently unknown. Such knowledge is important during artificial selection of grapevine varieties with best traits for controlling the disease through breeding of the virus-tolerant/resistant varieties. So far, no research project has officially released new breeding lines of grapevines which are resistant to the GLD in Tanzania (Tyagi *et al.,* 2022). Limited genetic resources among the cultivated germplasms of grapevines is among the major constraints to crop improvement by breeding despite lack of research funds and presence of insufficient, competent genetic breeders. By transcriptome analyses, different grapevine varieties were proved to exhibit distinct resistance and tolerance mechanisms against infection by different GLRaV species (Bester *et al.*, 2017). Equally, the proteomic studies are desirable for providing insights on protein-protein interactions between virus species and plant hosts (Gomaa *et al.*, 2024).

Forty-six (46%) and 24 (12%) of the suspected GLD-cases in the respective Chisichili and Mpunguzi villages, tested negative for the investigated 1, 2, 3, 4 and 7 types of the GLRaV species. This scenario could be associated with two reasons. Observational bias which is one constraint of the case-control design (Pandis, 2014) employed in this study could be the first reason why some of the grapevines with evident symptoms for the GLD tested negative for the GLRaV- 1, 2, 3, 4 and 7. Probably the GLD symptoms were confused with biotic stress such as nutritional deficiency (Magnesium and potassium) in the grapevines which triggers biosynthesis of flavonoids and cause reddening of leaves (Chooi *et al.*, 2022). The second reason could be that, the viruses were unequally distributed in the leaves and the virus titer in the analyzed tissue section was extremely low to be detected by the RT-PCR assay.

In addition to the GLD-outbreaks reported herein, persistent drought in Dodoma region (Lwelamira *et al.*, 2015) exacerbate the slow growth of wine industries and overall nation economy in the eastern African region. Drought characterized by high temperature cause accumulation of high sugar content in grape berries which may result to consequent production of grape wines with extremely high alcohol content (Olego *et al.*, 2016) above optimum range (9-15% ethanol per volume) (Lombardo *et al.*, 2023). Extremely high alcohol content poses detrimental heath effects to consumers, mainly gustatory disequilibrium which affects their sensory perception of wines (Jordão *et al.*, 2015). Production of wines with excessive alcohol content impose extra production costs to the wine manufacturing industries because different methods and technologies must be applied in order to reduce the undesirable alcohol levels and meet the required standard. For instance, genetically modified strains of yeast (*Saccharomyces cerevisiae*) have been used to control level of ethanol production during fermentation process of wine making. Such biotechnological strategy aims at conserving organoleptic properties, flavor and high quality of the beverage but there are consumers' concern regarding biosafety of wines produced by using the genetically modified products (Olego *et al.*, 2016). Wine manufacturing industries in Tanzania have not disclosed how they deal with grapes containing excessively high sugar concentration during the wine making process. In other instances, grapevines in fields are treated with growth regulators like the synthetic Auxin 1-naphthaleneacetic acid (NAA) in order to delay maturity and ripening onset of grape berries, as the hormone delay accumulation of sugars and anthocyanins in the fruits (Olego *et al.*, 2016). Unfortunately, Tanzanian farmers have not yet adopted such technology in viticulture.

CONCLUSION

The present study reports 54% and (82% and 6%) prevalence of respective GLRaV-3 and (3 and 1), the causal pathogens of the GLD outbreaks which emerged in the corresponding Chisichili (January 2024) and Mpunguzi (October 2023) villages, located in Dodoma, Tanzania. Outbreak of the GLD in Tanzania necessitates improvement of diagnosis, management and control of the disease for alleviation of the ascribed food insecurity, malnutrition and poverty. The governments of Tanzania and other Eastern African countries needs to ensure strict quarantine policies are implemented in order to inhibit exchange of grapevine planting materials across national borders in the zone. Furthermore, current farming and management practices of grapevines mainly phytosanitation measures need immediate improvement in order to reduce transmission of the GLRaV species between farms. That can be achieved through conduction of farmers' awareness campaigns on the disease epidemiology. Lastly, future research investments should be skewed to Omics-based (metatranscriptomics, proteomics and transcriptomics) and epigenetics studies of grapevines in order to address the following unanswered research questions: (i) What is the genetic diversity of the GRLaV species in uninvestigated grapevine producer villages (Mvumi, Hombolo, Chinangali, Mpunguzi, Mbabala A, Mbabala B, Mchemwa, Matumbulu and Veyula) in Dodoma and other regions (Morogoro, Kilimanjaro, Tanga, Tabora, Manyara (Babati), Mara (Bunda) and Ruvuma (Peramiho)in Tanzania? and (ii) What is the pathogenicity and virulent levels of the GLRaV strains circulating in Tanzania?

(iii) What is the current tolerance/resistance level of the grapevine germplasms against variants of the GLRaV species prevalent in Dodoma, Tanzania? Understanding the genetic structure of the GLRaV species will guide custom design of user friendly, highly sensitive and specific molecular assays for quick routine diagnosing the GLD. Furthermore, knowledge on virulence and pathogenicity levels of the GLRaV species prevailing in Tanzania as well as virus-resistance/tolerance levels in grapevines are essential for the disease control mainly production of virus-resistant grapevines through breeding and/or transgenesis by RNA interference technology for alleviation of food insecurity, malnutrition and poverty in East Africa.

ACKNOWLEDGEMENTS

The Grapevine farmers at Mpunguzi and Chisichili Villages in Dodoma, Tanzania whom participated in this study, are highly appreciated for their cooperation and valuable contributions.

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