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Zimbabwe is Free from Maize Chlorotic Mottle Virus (MCMV): The Chief Virus for Maize Lethal Necrosis

Mudada N[1,3*], Banana J[3], Tauya C[3], Sengwe T[2], Runyanga TJ[3], Nyaruwata K P[3], Makumbe L D M[3], Mujaju C[3], Kutywayo D[3], Mapope N[1] and Ngezimana W[1]

[1]Crop Science Department, Faculty of Plant and Animal Sciences, Marondera University of Agricultural Sciences and Technology. Cold Storage Company (CSC) Complex, Plot 15, Longlands Road, Marondera, P.O. Box 35, Marondera, Zimbabwe

[2]CIMMYT Zimbabwe, 12,5 km Peg, New Mazowe Road, Mazowe Zimbabwe

[3]Plant Quarantine Services Institute, 33 km Peg, Harare to Bindura Road, P. Bag 2007, Mazowe, Zimbabwe

ABSTRACT

Invasive cross-border plant pests such as Maize Chlorotic Mottle Virus (MCMV) introduced through pest-pathway exacerbated by anthropogenic behaviour negatively impact market access, food and nutrition security in Sub-Saharan Africa. Surveillance was carried out from 2020 to 2024 by conducting a census (100% population sampling) of maize pathways entering the country to check their MCMV pest-status and to ascertain the impact of pre-border plant biosecurity control measures in reducing accidental introduction of regulated pests. Double antibody-sandwich enzyme-linked immunosorbent assay (ELISA) and Bioreba-agristrips rapid test kits were used to test for the virus. A simple random sampling was used to pick maize fields for green tissues testing and maize grain and seeds from grain stores, seed stores and agro-dealers in the country. A symmetric analysis was computed to understand the association of samples originating from MCMV endemic and non-MCMV endemic areas Ordinal-by-Ordinal Associations test, (*Kendall's tau-b and tau-c = 0.333; Gamma = 0.333; Spearman's rho = 0.500; p = 1.000, Monte Carlo 99% confidence interval (CI): [1.000, 1.000*]) and Interval-by-Interval association and Chi –square Pearson's correlation coefficient (*Pearson's r = 0.996; at 99% CI of [0.321, 0.346*]) showed positive correlation which was not significant. The interval-level showed a close to perfect linear association between samples collected from MCMV endemic and non-MCV endemic countries. The Pearson's correlation X^2 (1); (p = 0.053) was also non-significant. A median (Kruskal–Wallis H) test showed no significant difference on the median values of MCMV endemic and non-MCV endemic (p = 0.223). There were no significance difference on the distribution of samples collected from endemic MCMV and none-endemic MCMV regions (α = 0.05; p = 0.368). All the tested 19106 samples were negative for MCMV. This research revealed that Zimbabwe is currently free from MCMV and it also confirmed the effectiveness of pre-border plant quarantine control measures in reducing the risk of germplasm as pest-pathways in cross border traffic particularly for the MCMV maize pathways approaching Zimbabwe.

***Corresponding author**

Mudada N, Crop Science Department, Faculty of Plant and Animal Sciences, Marondera University of Agricultural Sciences and Technology. Cold Storage Company (CSC) Complex, Plot 15, Longlands Road, Marondera, P.O. Box 35, Marondera, Zimbabwe.

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Introduction

Maize is a critical food security crop for Zimbabwe [1,2]. It is consumed by the majority of the population with an estimated per capita consumption of 110 kg per year [3]. Production of maize in Zimbabwe covers an area of approximately 1.2 million hectares. Mostly, the crop is produced by the small-holder and with the other components coming from large commercial farms. Over 80% of maize in Zimbabwe is produced by smallholder farmers for subsistence and livelihood. Zimbabwe is also currently one of the largest exporters of hybrid maize seed to markets in East Africa, Southern Africa and recently West Africa. Any disruption to maize production including maize seeds due to pests and other production constraints has impacts on the availability and price of maize grain and maize seed nationally and regionally.

Maize Lethal Necrosis (MLN) is caused by synergistic interaction between the Maize Chlorotic Mottle Virus (MCMV) from the genus *Machlomovirus* in the family *Tombusviridae* and any of the poty-viruses infecting cereals such as Sugarcane Mosaic Virus (SCMV), Wheat Streak Mosaic Virus (WSMV) and Maize Dwarf Mosaic Virus (MDMV) [4,5]. The diseases can also be caused by the co-infection of MCMV with other unrelated viruses [6,7].

In Africa, the disease was first reported in Kenya in Sept 2011 and the disease spread to Uganda, Tanzania, Rwanda, D.R. Congo, and Ethiopia by 2016 and currently Mozambique [8-12]. MCMV was first identified in Peru in 1973 and has been subsequently reported in the USA, parts of Latin America, and China, Kenya, Uganda, Tanzania, Democratic Republic of the Congo, South Sudan and Ethiopia. Globally, MLN has also been reported in Ecuador, and China (8,13,14,16,17). Maize plants are susceptible to MLN at all growth stages.

The diagnostic symptoms of MLN include chlorotic mottling of leaves, necrosis development from the leaf margin to the midrib, and dead heart; later-stage infection could lead to sterile pollen, small cobs with poor seed set, or death of the plants. Possible factors that contribute to the devastating effect of MLN include new and perhaps highly virulent strains of MCMV and SCMV, conducive environment for survival, proliferation and spread of insect-vectors of the viruses as well as continuous maize cropping in certain regions leading to build-up of virus inoculum [18].

One of the major duties of quarantine officials and pest risk assessment staff is to institute quarantine control measures as a legal method for pest and disease control. Quarantine is critical in making sure that the introduction of new pests is reduced through border control and the use of phytosanitary measures for imported consignments that could act as pathways for pests' introduction. It is very critical to note that surveillance is one of the key components for the determination of the pest status of a country or production site including the status of pathogens such as Maize Lethal Necrosis (MLN) causing viruses. This study determined the effects of pre -border plant quarantine control measures in reducing the risk of germplasm as pathways of pests spread using maize pest pathways as a case study for Zimbabwe.

Method

Site Selection and Sampling

Site

The surveillance covered 48 sites selected by the virtue of them being maize producing areas from the ten provinces of the country and the exit and entry points (Table 1). Cross-border maize pathways were sampled from all the official recognised Zimbabwe's ports of entries (Table 1) [19].

Table 1: Sites where Maize Samples were Collected for the Surveillance of MCMV in Zimbabwe from 2016 to 2022.

District / site	Border port
Guruve, Mazowe, Bindura, Shamva, Mt Darwin, Muzarabani, Mvurwi, Karoi, Mhangura, Chinhoyi, Banket, Kadoma, Kariba, Harare, Mhondoro, Shurugwi, Gokwe North, Gokwe South, Mvuma, Kwekwe, Beatrice, Chivhu, Chegutu, Gweru, Kadoma, Sanyati, Lupane, Bulawayo Umguza. Chiredzi, Zaka, Bikita, Gutu, Mrehwa, Mutoko, Macheke, Marondera, Goromonzi, Arcturus, Nyanga, Mutare, Rusape, Chipinge, Chimanimani, Beitbridge.	Mkumbura; Kariba, Chirundu, Robert Mugabe International Airport, Bulawayo Railway station, Bulawayo airport, Forbes, Nyamapanda, Sango, Beitbridge, Plumtree, Maitengwe, Kazungula and Mphengs.

Sampling Design

Simple random sampling was used for the collection of test samples from the growing maize crop. Sampling points were at least 20 km radius apart from each other. Green tissue for the maize grain and maize seed crops were randomly sampled from within the 45 survey sites from the eight provinces of the country.

The sampling of maize grain and maize seeds within the country also followed the simple random sampling technique where the samples were randomly drawn from maize grain storage centres from the Grain Marketing Board deports, farmers' grain stores, agro-dealer retail and wholesale markets, seed company depots and exit and entry border ports that were found within the 20 kilometre radius considered for the sampling of the maize green tissues. Despite following the random process to bet the seeds sampling points, the maize seed samples used in this research were purchased from retail markets within the 45 surveys centres described earlier to mimic the reality in the maize seed business. The free collection of maize seeds had to be avoided to any suspicion of bias on seed sample collection. The purchased of the seeds mimicked the environmental in which the farmers were mostly obtaining their seeds apart from using the retained seeds.

Sampling of maize germplasm and seeds at exits points was not random as it followed a census technique where all (100%) of the maize grain and seed pathways entering the country's borders were sampled for MCMV testing. All the maize grain (1169 consignments) and germplasm (17000 sets of germplasm) that passed the entry points into the country were sampled to test the status of MCMV. Sources of imported maize grain, seeds or germplasm were recorded as either endemic or non-endemic using the pest records report found in CABI data base or any other available sources including the internet search for MCMV presence reports [20,21].

Rapid Diagnostic Testing of MCMV using the Maize Green Tissues in the Field

A rapid testing approach was one of the techniques used during the surveillance of MCMV in Zimbabwe. Visual inspection of fields with growing maize crops was done at sites indicated in Table 1. From a field with an estimated area of about 10 hectares, six leaf samples were taken using the 'X' field inspection pattern from the CDFA Phytosanitary Certification Manual, 1985 (Figure 1). The six samples were randomly selected from farmers' fields within every 20km radius making an average total of 90 samples per region. This sample size was relatively maintained for the period of surveillance. For the testing of maize seed crops using the rapid diagnostic testing (RDT) technique, five (5) samples were randomly selected from seed production fields and maize agro-dealers making a total of 45 samples per each in all the regions.

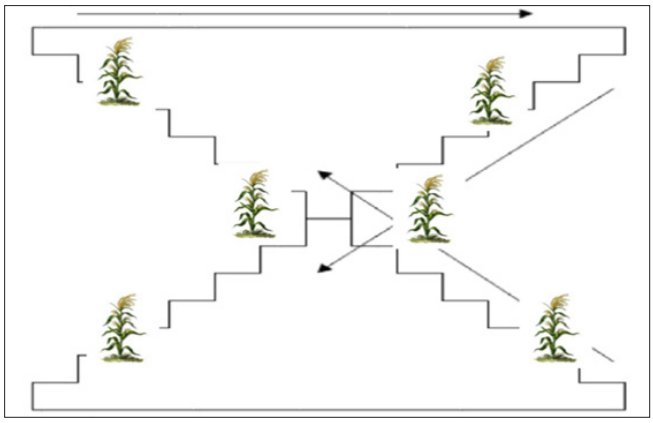

Figure 1: Staggered Sampling Adopted from CIMMYT MLN Protocol (2013)

The sampling procedure covered all parts of the field were adequately and proportionately represented for the plants inspected within the various usual microclimates of the field. Leaf samples taken were the youngest leaves of the maize plant and mostly, the flag leaf was sampled. Five to six-centimetre lengths segments of fresh leaf tissue were cut using bleach-cleaned scissors and

enclosed in a clean sheet of fresh tissue paper. Standard aseptic techniques were followed to avoid contamination.

The six leaf samples were placed singularly into an envelope at each site, the envelope was then perforated with air holes. Complete sample labels with a unique quick response (QR) code (DENSO International America, Inc. 1994) were stuck on each sample bag and the unique sample code was also put on each envelope with leaves. Each labelled sample bag was placed inside a medium zip-lock plastic bag. The six individually labelled leaf sample bags from the same sampling field were put inside one large zip-lock plastic bag (Bulk sample bag). Bulked six leaf samples from the sampling fields were immunostrip assayed together. Scissors and other implements were cleaned thoroughly with bleach solution between each sampling of a maize plant and test and between fields and between farms.

The bulked six leaves were cut into small pieces of ≈ 5 cm^2 and put into an extraction bag. Approximately 4 ml of Agri-Strip extraction buffer was added with a disposable pipette. The tissue was homogenized with a handheld homogenizer with a few movements for not more than 2 seconds. A drop of extract solution was transferred into a cuvette and diluted with 3 drops of extraction buffer. The end of the strip marked «sample» was inserted into the extract and the formation of coloured bends was observed. Results were recorded after 15 minutes. Positive results indicated two bands of colour changes whilst negative results indicated one line of colour change. The techniques with agri-strips testing used antibody and antigen reactions.

Testing of Maize and Grain Samples using Enzyme-Linked Immunosorbent Assay (ELISA)

Testing of maize and grain samples for MCMV followed the ELISA technique. The procedure similar to the one used by was used to test maize grain and germplasm. In this case, 100 µl of both test sample and positive control were dispensed into positive control wells, and 100 µl of sample extraction buffer was dispensed into buffer wells [22-25]. The plates were incubated inside humid box overnight in the refrigerator (4°C). 10 ml of the alkaline phosphatase enzyme conjugate was prepared by dispensing 10 ml of ECI buffer. Then, adding 50 µl from bottle with concentrated detection antibody and alkaline phosphatase enzyme conjugate and 50 µl from bottle alkaline phosphatase enzyme conjugate to the ECI buffer. The samples were washed with 1X PBST seven times. Exactly 100 µl of enzyme conjugate solution was dispensed per well. The plates were incubated in the humid box for 2 hours at room temperature. PNP solution was prepared by mixing 5 ml of room temperature 1X PNP buffer with one PNP tablet 15 minutes before the end of incubation time. Plates were washed 8 times with 1X PBST. 100 µl of PNP substrate was dispensed into each test-well. The plate was incubated for 60 minutes. Wells were examined by eyes and then ELISA reader. The wells were visual examined for colour change and also the plate was read using micro-titre plate ELISA-reader at 405 nm wavelength. All air bubbles present at the time if reading were eliminated to avoid wrong readings due to interference of the reader-light path. Wells in which colour changes to yellow indicated positive results while wells in which there was no significant colour development indicated negative result. Test results were considered valid only if positive control wells gave a positive result while negative control and buffer wells remain colourless or gave negative results.

Construction of Geospatial Maps of the Sampling Sites

GPS coordinates were collected from maize, green tissue, grain centres and maize seeds retail outlets sampling points and these were subjected to spatial data maps using free GPS tools. ArcGIS was used to construct the geospatial data.

Data Analysis

Data collected from the survey was coded and analysed for both descriptive and quantitative statistics using Statistical Package for Social Sciences (SPSS) Version 16.0. Spatial maps were drawn using the GPS coordinates of the data collection sites. A chi-square square Pearson correlation coefficient, Spearman's correlation, Goodman and Kruskal's gamma coefficient were used as symmetric (directional) measures to describe the relationships between the independent (antecedent) and dependent (consequent) variables. The Kruskal-Wallis H test was computed as an alternative to one-way ANOVA where the assumption of normality was violated by checking statistically significant difference between the medians. Asymmetric measures were also applied where applicable, to determine association coefficients of the independent and none independent variables [26,27]. For multivariate cases, canonical correlation analysis was used for two variable sets and a multiple correlation coefficient was used for sets of variables. Histograms were the visual methods used to check for data normalcy.

Results

Introduced Maize Germplasm and Grain

As shown in Table 2 and 3, 1169 introduced maize grain samples from both MCMV endemic (111 samples) and non-MCMV endemic (1058 samples) countries tested negative for the pest using ELISA. The ELISA testing showed yellow-coloured bands with reading ranging from 0.4 to 0.415 for the positive control samples, whilst the test samples showed no colour changes and displayed readings ranging from 0.091 to 0.277. The negative control samples had no colour changes and they displayed readings ranging from 0.244 to 0.2445. Both the negative control and test samples had no MCMV antibodies (Figure 2).

Table 2: Introduced Maize Grain Pest-Pathways Sampled and tested for Maize Chlorotic Mottle Virus (MCMV) During the Period 2020 – 2024 in Zimbabwe

Year	Number of maize grain samples originating from MCMV endemic areas.	Number of samples originating from non-MCMV endemic areas	Total Number of samples tested.	Rapid Detection Test result using bopreba-agristrips.	Enzyme Linked Immunosorbent Assay Test Result
2020	6	168	174	Negative	Negative
2021	67	438	505	Negative	Negative
2022	38	452	490	Negative	Negative
Total	111	1058	1169	Negative	Negative

Table 3: Introduced Maize Germplasm Screened at the Mazowe Plant Quarantine Services Institute Maize Lethal Necrosis Screening Facility for Maize Chlorotic Mottle Virus (MCMV) from 2020 to 2024 in Zimbabwe

Year	Number of germplasm lines introduced	MLN RDT test result during vegetative growth	ELISA test Results of the harvested seeds
2020	4900	Negative	Negative
2021	5800	Negative	Negative
2022	6300	Negative	Negative
Total	17000		

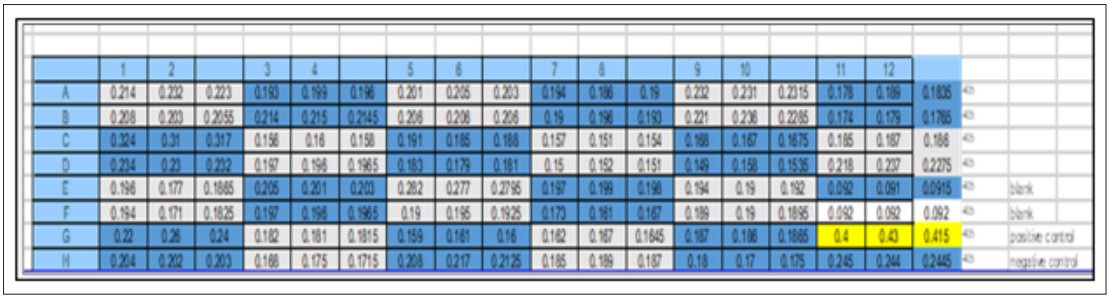

Figure 2: Optical density from ELISA Reader Obtained During the MCMV Testing during 2020 to 2024 Maize Chlorotic Mottle Virus Screening in Zimbabwe

A symmetric analysis was computed to understand the association of samples originating from MCMV endemic areas and those originating from non-MCMV endemic areas from 2020 to 2024. Nominal-by-nominal test indicated high association coefficients which were not significant (Phi = 1.414; Cramer's V = 1.000; and Contingency Coefficient = 0.816; Monte Carlo simulation testing (p = 1.000, 99% confidence interval (CI): [1.000, 1.000]), Table 4. Ordinal-by-Ordinal Associations showed moderately positive correlations which were also not significant (Kendall's tau-b and tau-c = 0.333; Gamma = 0.333; Spearman's rho = 0.500; p = 1.000, Monte Carlo 99% CI: [1.000, 1.000]). Interval-by-Interval association and chi –square Pearson's correlation coefficient also revealed very high associations (Pearson's r = 0.996; at 99% CI of [0.321, 0.346]) which were as well not significant. The interval-level showed a close to perfect linear association between samples collected from MCMV endemic and non-MCV endemic countries. The Pearson's correlation X^2 (1); (p = 0.053) was non-significant. A median (Kruskal–Wallis H) test showed no significant difference on the median values of MCMV endemic and non-MCV endemic (p = 0.223). There were also no significance difference on the distribution of samples collected from endemic MCMV and none-endemic MCMV regions (α = 0.05; p-value: 0.368).

Table 4: The SPSS Statistical Output of the Symmetric Analysis of the Association of Samples Originating from MCMV Endemic Areas and those Originating from non-MCMV Endemic Areas Imported Maize Grains Sampled from all Entry Points in Zimbabwe

		Value	Asymptotic Standard Error	Approximate Tb	Approximate Significance	Monte Carlo Significance		
						Significance	99% Confidence Interval (CI	
							Lower Bound	Lower Bound
Nominal by Nominal	**Phi**	1.414	N/A	N/A	0.199	1.00[c]	1.000	1.000
	Cramer's V	1.000	N/A	N/A	0.199	1.00[c]	1.000	1.000
	Contingency Coefficient	0.816	N/A	N/A	0.199	1.00[c]	1.000	1.000
Ordinal by Ordinal	**Kendall's tau-b**	0.333	0.544	0.612	0.540	1.00[c]	1.000	1.000
	Kendall's tau-c	0.333	0.544	0.612	0.540	1.00[c]	1.000	1.000
	Gamma	0.333	0.544	0.612	0.540	1.00[c]	1.000	1.000
	Spearman Correlation	0.500	0.612	0.577	0.667[d]	1.00[c]	1.000	1.000
Interval by Interval	**Pearson's R**	0.996	0.003	11.894	0.053[d]	0.334[c]	0.321	0.346
Measure of Agreement	**Kappa**	0.000	0.000			0.0[c]		
N of Valid Cases (years)		3						

Locally Sampled Maize

Maize Green Tissue

As shown Table 5, the results of the testing of maize green tissue from the growing maize crop sampled from Zimbabwe's maize production areas during the period of this study. As shown in Table 5, all the 531 maize grain tissues samples tested produced negative result for MCMV using Bioreba-agristrips rapid detection test (RDT). The tests carried out for the leaf samples, produced a control band that showed absence of the MCMV (Figure 2).

Table 5: Green Leaf Tissue Tested for MCMV from Growing Maize Crops from 2020 to 2024 in Zimbabwe

Year	Number of samples tested	MCMV RDT result	ELISA test Results
2022	100	Negative	Negative
2023	233	Negative	Negative
2024	198	Negative	Negative
Total	531		

Maize seed and grain

As shown in Table 6, all the 406 samples tested negative to MCMV using both Bioreba-agristrips and ELISA.

Table 6: Locally Collected Maize Grain and Seed Samples Tested for MCMV from the 45 Survey sites in Zimbabwe from 2020-24

Year	Number of maize grain samples collected and tested.	Number of maize seed samples purchased and tested	Total Number of samples tested.	Rapid Detection Test result using Bioreba-agristrips.	Enzyme Linked Immunosorbent Assay Test Result
2020	81	13	94	Negative	Negative
2021	62	11	73	Negative	Negative
2022	34	11	45	Negative	Negative
2023	53	19	72	Negative	Negative
2024	95	27	122	Negative	Negative
Total	325	81	406	Negative	Negative

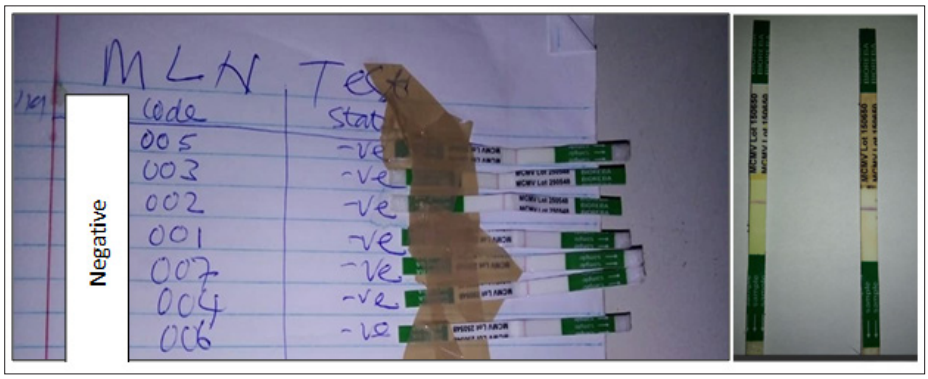

Figure 3: Negative Immunostrip Tests with One Red Band showing Negative Result for MCMV during the Period 2020-2024 in Zimbabwe

Other Results

Physiological Disorders

During the field sampling of green maize crops, symptoms of nutrient deficiency were observed on leaves of maize crops growing in the fields of small-scale farms.

Other Pathological Diseases

Symptoms of the Maize Streak Virus (MSV) were observed during the survey. Grey leaf spot, Curvularia and leaf blight were fungal symptoms detected during the survey. Furthermore, the symptoms of the common rust, corn smut, and Phaesphaeria leaf spot, northern corn leaf blight were also observed. Maize streak virus and common rust were the major diseases found in growing maize crop from this current research. For these specified diseases, the disease incidence ranged from 2- 15% and averaged 3%.

Insect Pest Incidence

Insects observed during the field inspections in the growing maize crops included aphids, leaf beetles, whiteflies, fall armyworms and maize stalk borers, webworms and weevils. Figure 3 show the sampling sited for the seeds, and growing maize during the MCMV surveillance in Zimbabwe.

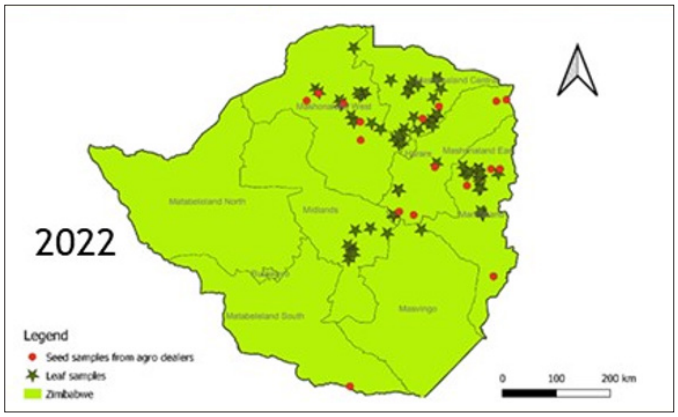

Figure 3: Sampling Sites for the growing Maize Crop Leaf Samples for the MCMV Testing in 2022 in Zimbabwe (Maps Drawn by CIMMYT, [22].)

Production Information

Crop stages of the maize crop that were considered for MCMV green tissue sample testing for MCMV were at the vegetative (VE-VT) and reproductive (Silk, Blister, Milk, Dough, Dent, and Maturity) stages. The surveys sampled fewer crops in VE-VT stage as compared to the other stages. The time of planting ranged from October to January in each year from 2020 to 2024. Commercial seed farmers supplemented the water requirements with irrigation and hence planted the maize crops earlier in October. Small-scale farmers planted with the rainfall from October to January depending on the onset of the rainfall season. Seeds were sourced from seed houses and or retained seeds and the varieties included both hybrids and open-pollinated ones.

Discussion

Data from this present study revealed the absence of MCMV in Zimbabwe as observed from other countries which used the same MCMV protocol adopted from CIMMYT [28]. The absence of MCMV could be a positive result for the country's maize value chain. The absence of MCMV for country aligned to the country's desired phytosanitary status of food security as well as being a hub of maize seed trade in the region. Zimbabwe processed seed maize phytosanitary certificates for export into the regional and COMESA countries. The result of this current research are similar to what was obtained in Zambia where MCMV was found absent [24]. In contrast, this kind of research revealed positive record of MCMV in Mozambique, Tanzania, Kenya, Somalia and Ethiopia [8,25,22].

Research in US and Kenya showed that insect vector control, crop rotation, and crop diversification are among the agronomic practices that play an important role in preventing or reducing the risk of diseases [22]. From this current research, the practice of monoculture was observed. Mono-culturing maize crop can lead to high disease build-up as in tobacco production (Waithira, 2023). even though no MCMV pathogen was detected in the sampled maize products, the fear for the virus remained high due to porous border especially given the detection of maize lethal necrosis in the neighbouring Mozambique [12]. The proximity of the reporting of the MLN causing virus in the neighbouring Mozambique with a relatively free movement of people across the porous borders is likely to threaten the country biosecurity.

Even though studies have shown low rates of seed transmission, these low rates of seed transmission are significant to cause a pandemic especially with vectors coming in to equation where they play larger roles in disease dissemination [6]. Zimbabwe has aphids, thrips, and beetles which are vectors of MLN and their presence suggests a time bomb in cases of invasion by MCVMV. Epidemics can occur very quickly through infected seed. MCMV was reported in Tanzania and Democratic Republic of Congo [28]. Given the increased trade amongst these states with Zimbabwe, plant biosecurity mechanism at ports of entry requires strengthening. Probably by reinforcing and instituting entire preventive and control measures, to kept the disease out of her borders. Even though Zimbabwe was free from MCMV during this research, continued institution of quarantine measures for MCMV remains valid for the maintenance of its MCMV biosecurity status. The presence of SCMV puts Zimbabwe at risk to a serious epidemic once the MCMV is accidental introduced into the country [32].

A similar study in the United States of America and Kenya revealed that insect vector control, crop rotation, and crop diversification are amongst the agronomic practices that play an important role in preventing or reducing the risk of maize lethal necrosis diseases [33,34]. Furthermore it has been shown in Kenya that effective monitoring, and rigorous implementation of maize-free periods and rotation with non-cereal crops had helped that country to minimizing MLN incidence [34]. Although the use of dead season might be a good phytosanitary strategy to mitigate the challenge of the disease as was done in Kenya, the absence of a pest in a geographic area remains a critical answer to challenges associated with invasive pest species in plant biosecurity control [35].

Data from this present study revealed moderate associations ($p = 1.000$, Monte Carlo 99% CI: [1.000, 1.000]) on samples originating from MCMV endemic areas and those originating from non-MCMV endemic areas, the lack of significance differences between the two geographic areas indicated a strong measures on the quality of phytosanitary pre-border plant biosecurity management practices from the exporting countries. In contrast, several symmetric measures reported strong or moderate associations across different variable types, none of the associations were statistically significant, further strengthening the importance of pre-border pest management practises in cross border pest pathways approaching Zimbabwe.

Conclusion

From this current study, Zimbabwe is free from Maize Chlorotic Virus and the received germplasm from MCMV endemic countries were found free from the virus hence suggesting the effectiveness of pre-border phytosanitary measures in reducing the risk of germplasm as pest-pathways for maize chlorotic mottle virus (MCMV) approaching Zimbabwe from endemic regions.

Recommendations

Yearly surveillance of MLN causing viruses is critical for quick and early detection.

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References

1. Kahiya C (2022) Zimbabwean Food: 17 Popular and Traditional Dishes to Try. Nomad Paradise [Internet]. Available from: https://nomadparadise.com/zimbabwean-food/#.
2. Anonymous (2013) Zimbabwe Agenda for Sustainable Socio-Economic Transformation (Zim Asset), Towards an Empowered Society and a Growing Economy. Zimbabwe Government zim.gov.zw. Government zim.gov.zw.; 2013.
3. Esterhuizen (2018) The supply and demand of corn feed in Zimbabwe, Global agricultural information USDA foreign Agriculture Services [Internet]. Available from: https://apps.fas.usda.gov/.
4. Mezzalama M, Prasanna DB BM (2015) MLN Free Seed Production and Safe Exchange to Non-endemic Countries. D.F.: CIMMYT; Mexico: 2015. MLN pathogen diagnosis. [Google Scholar]. CIMMYT Mex.
5. Williams L, Alexander L (1965) Maize dwarf mosaic, a new corn disease. Phytopathology 55: 802-804.
6. Mahuku G, Lockhart BE, Wanjala B, Jones MW, Kimunye JN, et al. (2015) Maize Lethal Necrosis (MLN), an Emerging Threat to Maize-Based Food Security in Sub-Saharan Africa. Phytopathology®. 105: 956-965.
7. Redinbaugh MG, Zambrano JL (2014) Control of Virus Diseases in Maize. In: Advances in Virus Research [Internet]. Elsevier 391-429.
8. Wangai AW, Redinbaugh MG, Kinyua ZM, Miano DW, Leley PK, et al. (2012) First Report of Maize chlorotic mottle virusand Maize Lethal Necrosis in Kenya. Plant Dis 96: 1582-1582.
9. Regassa B, Abraham A, Fininsa C, Wegary D (2021) Alternate hosts and seed transmission of maize lethal necrosis in Ethiopia. J Phytopathol 169: 303-315.
10. Mwatuni FM, Nyende AB, Njuguna J, Xiong Z, Machuka E, et al. (2020) Occurrence, genetic diversity, and recombination of maize lethal necrosis disease-causing viruses in Kenya. Virus Res 286: 198081.
11. Frank K, Robert G, Brian EI (2025) Status of maize lethal necrosis in eastern Uganda. Afr J Agric Res 11: 652–660.
12. Stewart L (2022) Maize lethal necrosis. 119663. Available from: http://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.119663.
13. Castillo LJ (1977) Maize virus and virus-like diseases in Peru. Proceedings of the International Maize Virus Disease. Colloq Workshop Wooster Ohio USA. 27: 363-369.
14. Xia Z, Zhao Z, Chen L, Li M, Zhou T, et al. (2016) Synergistic infection of two viruses MCMV and SCMV increases the accumulations of both MCMV and MCMV-derived siRNAs in maize 6: 20520.
15. Adams IP, Harju VA, Hodges T, Hany U, Skelton A, et al. (2014) First report of maize lethal necrosis disease in Rwanda. New Dis Rep 29: 22-22.
16. Quito-Avila D, Alvarez R, Mendoza A (2016) Occurrence of maize lethal necrosis in Ecuador. A disease without boundaries?. Eur J Plant Pathol 146: 705-710.
17. Rao Y, You Y, Zhu S, Yan J, Huang G, et al. (2010) The loss evaluation index system and the direct economic losses of Maize chlorotic mottle virus invading China. Plant Quar 24: 5-8.
18. Cabanas D, Watanabe S, Higashi CHV, Bressan A (2013) Dissecting the Mode of Maize Chlorotic Mottle Virus Transmission (Tombusviridae: Machlomovirus) by Frankliniella williamsi (Thysanoptera: Thripidae). J Econ Entomol [Internet] 106: 16-24.
19. Customs and Excise Act (CAP 23:02). 1974.
20. CABI. Crop Protection Compendium. CD-ROM.; 2005.
21. CABI (2025) Crop Protection Compendium. CAB International, Wallingford. https://www.cabi.org/PRA-tool [Internet]. 2025. Available from: https://www.cabi.org/PRA-tool
22. Mahabaleswara SL, Hodson DP, Alemayehu Y, Mwatuni F, Macharia I, et al. (2024) Monitoring and tackling Maize Lethal Necrosis (MLN) in Eastern and Southern Africa from 2014 to 2024. CIMMYT. In Kenya: CIMMYT; 2024. Available from: https://repository.cimmyt.org/entities/publication/fc627780-4894-4990-a83d-1c84d6e51b0c
23. Prassana B (2021) Maize Lethal Necrosis (MLN): A Technical Manual for Disease Management. [Internet]. Nairobi, Kenya: CIMMYT; 2021. Available from: CIMMYT https://repository.cimmyt.org/entities/publication/31d778db-9030-4cf7-9e77-04e70742e815.
24. Chomba D, Msiska K, Abass M, Mudenda M, Mukuwa P (2020) Status of Maize Lethal Necrosis Disease in Zambia. Afr Phytosanitary J 2: 1-16.
25. Frank K, Robert G, Brian EI (2016) Status of maize lethal necrosis in eastern Uganda. Afr J Agric Res 11: 652-660.
26. Kendall MG (1962) Rank Correlation Methods [Internet]. Hafner Publishing Company; (Griffin books on statistics). Available from: https://books.google.co.zw/books?id=1whKAAAAMAAJ.
27. Association (2004) In: M S Lewis-Beck, A Bryman, T Futing Liao (Eds) The SAGE Encyclopedia of Social Science Research Methods [Internet]. 2455 Teller Road, Thousand Oaks California 91320 United States of America: Sage Publications 29-29.
28. Boddupalli P, Suresh LM, Mwatuni F, Beyene Y, Makumbi D, et al. (2020) Maize lethal necrosis (MLN): Efforts toward containing the spread and impact of a devastating transboundary disease in sub-Saharan Africa. Virus Res 282: 197943.
29. Waithira J (2023) Status of Maize Lethal Necrosis in Seed Production Systems and Interaction of Viruses Causing the Disease in Kenya [Thesis, University of Nairobi]. [Internet]. [Kenya, Nairobi]: University of Nairobi; Available from: http://erepository.uonbi.ac.ke/handle/11295/164469
30. Pratt CF, Constantine KL, Murphy ST (2017) Economic impacts of invasive alien species on African smallholder livelihoods. Glob Food Secur 14: 31-37.
31. Mezzalama M, Prasanna B BM (2015) MLN pathogen diagnosis, MLN-free seed production and safe exchange to non-endemic countries.https://repository.cimmyt.org/server/api/core/bitstreams/7cc635dc-5e71-4e6c-890b-82b528371986/content. CIMMYT brochure Mexico DF: CIMMYT; 2015.
32. Plant Quarantine Services Institute (2023) Annual report 2023. Mazowe, Zimbabwe: Plant Quarantine Services Institute, Department of Research and Specialist Services, Mazowe, Zimbabwe.
33. Kimani EN, Micheni CM, Muriki LG, Kiarie SM, Miano DW, et al. (2024) Influence of genotypes and crop growth stage at time of infection on seed transmission of Maize chlorotic mottle virus. Arch Phytopathol Plant Prot 57: 95-111.
34. Kimani EN, Kiarie SM, Micheni C, Muriki LG, Miano DW, et al. (2021) Maize Seed Contamination and Seed Transmission of Maize Chlorotic Mottle Virus in Kenya. Plant Health Prog 22: 496-502.
35. Mudada N, Mapope N, Ngezimana W (2022) The threat of transboundary plant pathogens to agricultural trade in Southern Africa: a perspective on Zimbabwe's plant biosecurity – A review. Plant Pathol Quar 12: 1-33.