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Early Symptom Characterisation and Mitigation of Maize Lethal Necrosis Disease

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ABSTRACT

Maize (*Zea mays*) is a staple crop central to food security and livelihoods across sub-Saharan Africa. In recent years, maize production in the region has been severely affected by maize lethal necrosis disease (MLND). First reported in Kenya in 2011, MLND has spread rapidly across eastern and central Africa, leading to significant yield reductions and serious socioeconomic impacts on smallholder farmers. The study examined early symptom development of MLND and evaluated the effects of foliar boron and exogenous RNase A on the growth of maize plants inoculated with MLND-infected sap. Two completely randomised trials were conducted 1 week after inoculation to assess maize growth responses to varying foliar boron concentrations and exogenous RNase A over 14 days. Stem inoculation induced a progressive sequence of foliar symptoms 2 days postinoculation, from discrete lesions and faint chlorotic streaks to extensive mosaic yellowing and tissue collapse. Advanced infection caused apical meristem death in 28.9% of the inoculated plants, triggering compensatory lateral shoot growth. Foliar application of exogenous RNase A resulted in statistically similar ($p > 0.05$) growth traits when compared to the inoculated plants with no treatments. However, foliar application of boron at a concentration of 15 mg L^{-1} resulted in significantly higher ($p < 0.05$) aboveground biomass yield when compared to the inoculated plants with no treatments. Molecular and serological analyses show that boron application at 15 mg L^{-1} effectively suppressed viral accumulation to below detectable levels. These findings provide a basis for developing low-cost strategies to manage the disease.

1 | Introduction

Africa's food security largely depends on maize (*Zea mays*), which contributes approximately 25% of dietary energy intake in eastern Africa and supports the livelihoods of millions of smallholder farmers (Biswal et al. 2022; Flett and Mashingaidze 2016). Despite its importance, maize productivity in sub-Saharan Africa (SSA) remains low (about 1.7 tons hectare^{-1}) compared to the global average (approximately 5 tons hectare^{-1}), largely due to abiotic and biotic constraints, including emerging diseases (Boddupalli et al. 2020).

Maize lethal necrosis disease (MLND) has recently emerged as one of the most devastating threats to maize production in SSA (Zhan et al. 2022). MLND is caused by the co-infection of maize chlorotic mottle virus (MCMV) of the family *Tombusviridae* and one of several cereal-infecting viruses in the *Potyviridae* family, such as sugarcane mosaic virus (SCMV), maize dwarf mosaic virus (MDMV), Johnsongrass mosaic virus (JGMV), and wheat streak mosaic virus (WSMV) (Adams et al. 2013; Goldberg and Brakke 1987; Scheets 1998; Stewart et al. 2017). The first MLND outbreak in Africa was reported in 2011 in East Africa, along Kenya's Rift Valley

Region (Wangai et al. 2012). Within 3 years (2012–2015), the disease was confirmed in Uganda, Rwanda, DRC Congo, Tanzania, Ethiopia and South Sudan (Awata et al. 2019; Lukanda et al. 2014; Mahuku et al. 2015; Redinbaugh and Stewart 2018). The disease is causing yield losses of up to 90% and significant economic damage to smallholder farming systems (Mahuku et al. 2015; Pratt et al. 2017).

MLND symptoms are more severe than those caused by individual viral infections and include chlorotic mottling, progressive leaf necrosis and premature plant death (Niblett and Claflin 1978; Uyemoto et al. 1981; Zhan et al. 2022). The disease is primarily spread by insect vectors such as thrips, and its incidence is exacerbated by continuous maize cultivation, which provides a persistent viral reservoir (Awata et al. 2019; Cabanas et al. 2013; Mahuku et al. 2015; Mwando et al. 2018). Numerous techniques, including symptomatology, serological approaches, nucleic acid-based procedures and electron microscopy, can be employed to diagnose MLND (Mekureyaw 2017). Practical approaches based on symptom observation, serology, and nucleic acids have been widely used (Zhan et al. 2022). Accurate field identification of MLND symptoms by farmers plays a key role in preventing the spread of the disease, as it enables the timely removal of infected plants and implementation of vector-control measures through pesticide application (Kiruwa et al. 2019).

Short-term strategies like crop rotation and maize-free windows are considered particularly effective in controlling MLND (Marenya et al. 2018). Integrated pest management (IPM) has been reported as the best choice in controlling and managing MLND (Boddupalli et al. 2020; Kiruwa et al. 2016; Mekureyaw 2017; Redinbaugh and Stewart 2018). Effective IPM for MLND includes strengthening detection, focusing on prevention and effective control (sanitation, crop rotation, chemical treatments and use of tolerant or resistant varieties) of the disease (Boddupalli et al. 2020; Zhan et al. 2022).

MLND is caused by RNA viruses whose successful infection depends on efficient replication and systemic movement through host tissues (Carino et al. 2020; Scheets et al. 1993). Enhancing host structural integrity and antiviral defence mechanisms may reduce disease severity. Boron is an essential micronutrient that is frequently deficient in agricultural soils and has been shown to reduce plant susceptibility to a wide range of diseases by influencing cell wall structure, cell membrane stability and lignin and phenolic metabolism (Blevins and Lukaszewski 1998; Brown et al. 2002). Adequate boron nutrition has been associated with reduced severity of several viral and fungal diseases (Graham et al. 1991; Marschner 1995; Rolshausen and Gubler 2005). Similarly, RNases are RNA-degrading enzymes involved in various cellular processes such as stress responses and plant defence (Hugot et al. 2002; Singh et al. 2020). Increased RNase activity has been reported following viral infection, wounding and induction of systemic acquired resistance, suggesting a role in antiviral defence (Lers et al. 1998; Lusso and Kuc 1995). Moreover, enhanced resistance to RNA viruses has been observed in plants expressing heterologous RNase genes or associated with RNase-producing endophytes (Burkhanova et al. 2019; Sugawara et al. 2016).

Given these roles, boron supplementation and exogenous RNase A application were hypothesised to mitigate MLND symptom development. This study had two main objectives: (1) monitor and characterise the early development and progression of symptoms associated with MLND in Tanzania; and (2) assess the potential of exogenous RNase A and boron treatments in symptom severity mitigation and on the growth parameters of the MLND-infected plants. The findings may provide insights into practical, field-applicable strategies for improving MLND management in SSA.

2 | Materials and Methods

2.1 | Plant Materials and Growing Conditions

This study was conducted between August 2025 and January 2026 in a screenhouse at The Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania (3.400278S and 36.799167E). The cultivar used for this study was *Zea mays* cv. AMH 501. Seeds were planted in 10 L buckets filled with soil, with one seed per bucket, evenly spaced at 76.2 cm x 76.2 cm, following the application of 6.5 g diammonium phosphate (DAP) fertiliser (18% nitrogen and 46% phosphorus) (Tanzania Fertiliser Company Limited, Dar es Salaam, Tanzania). Water was added occasionally, depending on the soil moisture content, to maintain adequate conditions for germination. Seeds germinated 7 days after planting. The seedlings were subsequently watered every 48 h for one additional week, and 6.5 g NPK fertiliser (Falcon Fertilisers, Dar es Salaam, Tanzania) was applied using the deep placement method before inoculation. The initial and final light intensities were 945.6 ± 90.0 and $1082.6 \pm 64.6\,\mu mol\,m^{-2}\,s^{-1}$ (Quantum PAR Meter, Gain Express Holdings Ltd., Hong Kong, China), respectively. The initial environmental CO_2, temperature and relative humidity in the screenhouse were 451.7 ± 17.6 ppm, $31°C \pm 4°C$, and $38\% \pm 9.5\%$, respectively. The final environmental CO_2, temperature and relative humidity in the screenhouse were 483.3 ± 62.0 ppm, $36.3°C \pm 6.4°C$ and $26.0\% \pm 7.0\%$, respectively.

2.2 | Inoculation

Leaf samples were collected from nearby field-grown maize plants showing symptoms consistent with MLND (Figure 1) (Arusha, Tanzania), as previously described in the literature (Hussein 2017; Suresh 2019; Terefe and Gudero 2019; Wangai et al. 2012; Zhan et al. 2022). The collected leaf samples were homogenised using a mortar and a pestle with tap water to create a sap. Using a needle-laden syringe, 2.0 mL sap was injected into the stem vasculature of each maize plant at 7 days after germination. The same inoculum preparation was used for all plants to maintain consistency in inoculum source across treatments. Plants were monitored daily for symptom development following inoculation with sap from a maize plant infected with MLND. Characteristic foliar symptoms were recorded and photographed (Figure 2). These symptoms were used as a visual confirmation of possible MLND in inoculated plants before initiating treatment.

FIGURE 1 | Field-grown maize plant showing symptoms consistent with MLND, used as an inoculum source.

2.3 | Molecular and Serological Detection of MLND

Maize leaf samples were preserved with ice-packs and sent to Egerton University.

(Nakuru, Kenya) for molecular and serological analysis. Total nucleic acid (deoxyribonucleic acid and ribonucleic acid) was extracted from the maize leaf samples using a modified cetyltrimethylammonium bromide (CTAB) protocol, where 0.4 g of maize leaf tissue was ground in 2 mL of extraction buffer using a mortar and pestle (Semagn 2013). The RNA pellet was suspended in 50 μL of deionised water. Agarose gel electrophoresis was conducted to evaluate the integrity and quality of the extracted total nucleic acids. Complementary DNA (cDNA) was prepared from 1 μg of RNA using first Strand cDNA Synthesis Standard Protocol (New England BioLabs, Ipswich, USA) as per the instruction manual. The synthesised cDNA was used as a template for polymerase chain reaction (PCR) with MCMV and SCMV-specific primers. MCMV-specific primers used were MCMV F 5′-AACATTCACAGCAGACACC-3′ and MCMV R 5′-GATAGCCACAATGAATCGTCC-3′, whereas SCMV-specific primers were SCMV F 5′-TCTACTGAGCGATACATGCC-3′ and SCMV R 5′-CGTGTGTTTGAACCACGAAC-3′ to produce an amplicon of 259 and 169 bp in length, respectively. The PCR conditions for MCMV reaction were 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 50°C for 30 s and 72°C for 1 min, with a

FIGURE 2 | Symptoms in maize plants 7 days after inoculation, used as a visual confirmation of infection before initiating treatment.

final extension at 72°C for 7 min. The PCR conditions for SCMV reaction were similar to those of MCMV but with the annealing temperature set at 60°C.

Serological detection was conducted for confirmation of PCR results for MCMV using MCMV AgriStrip (BIOREBA AG, Christoph Merian-Ring 7, CH-4153 Reinach BL1, Switzerland) following the manufacturer's instructions.

2.4 | Experimental Treatments

Experimental treatments were initiated 7 days postinoculation, once symptoms were visually confirmed in all the inoculated plants. Boric acid (Fisher Scientific, Ottawa, Canada) and RNase A from bovine pancreas (Millipore Sigma, Ontario, Canada) were used as the source of boron and exogenous RNase A for the study, respectively. Boron was applied alone at three concentrations [5 mg L^{-1} (B5), 10 mg L^{-1} (B10), and 15 mg L^{-1} (B15)], while RNase A was applied as a separate treatment at 12 μg mL^{-1} (RNase-12). Combined boron and RNase A treatments were not included in this study. All treatments were applied as foliar sprays every 48 h for 14 d (Table 1). The one treatment level of RNase A was selected based on a previous study that shows that 12 μg mL^{-1} of exogenous RNase A produced the most pronounced improvements in hop latent viroid (HLVd)-infected cannabis plant (unpublished data). An inoculated group with no treatment (NT) and an uninoculated group with no treatment served as the control plants for the study. The control plants were used to evaluate the effect of boron and RNase A. Water was applied to the control plants through foliar spray every 48 h for 14 d to match the treatment conditions.

All experimental procedures were carried out in a screenhouse that limited insect entry and minimised the risk of vector-mediated pathogen spread. To further prevent cross-contamination between treatments, uninoculated control plants were placed 3 m away from the inoculated group. Additionally, a physical barrier made up of 12 uninoculated maize plants arranged in two rows was set between treatment groups to act as a biological fence.

TABLE 1 | Experimental treatments.

Treatments	Boron (mg L^{-1})	Exogenous RNase A (μg mL^{-1})	Number of plants per treatment
Uninoculated (Control)	—	—	3
Inoculated (NT)	—	—	3
Inoculated (B5)	5	—	3
Inoculated (B10)	10	—	3
Inoculated (B15)	15	—	3
Inoculated (RNase-12)	—	12	3

2.5 | Plant Growth Parameters

Data collection for growth parameters commenced at the onset of treatment application (7 days post-inoculation), once symptoms were visually confirmed, and continued until the end of the treatment period (21 days postinoculation). Final measurements were taken at harvest following completion of the 14-day treatment period. Plant growth parameters, including height, stem diameter, fresh mass and dry mass, were measured. Plant height was measured from the top of the soil at the base of the plant to the tip of the uppermost leaf (tassel). Stem diameter was measured 5 cm from the top of the soil using a carbon fibre composite digital calliper (Dasqua 2220–8113, Chengdu, Sichuan, China). Shoot mass was weighed using an OHAUS Explorer Precision Balance (OHAUS Corporation, New Jersey, USA) to determine aboveground fresh and dry mass. Dry masses were measured after the shoot samples were left in the screenhouse in a temperature range of 35°C–40°C for 2 weeks.

2.6 | Statistical Analysis

This study comprised two independent trials, each arranged in a completely randomised design (CRD). Each treatment group consisted of 3 plants per replicate, and the experiment was repeated three times, resulting in a total of 9 plants per treatment. Data were statistically analysed using JMP software (JMP 4.3, SAS Institute Inc.) to investigate the impact of boron concentrations and exogenous RNase A dosage on the growth traits of MLND-infected maize plants. Mean values and standard deviation were determined with Microsoft Excel (Microsoft, Redmond, Washington, USA). A one-way ANOVA was conducted at a 95% confidence level ($p < 0.05$), and differences among treatment means were determined using the Tukey–Kramer significant difference test.

3 | Results

This study aimed to characterise the development and progression of early symptoms associated with MLND-infected maize plants in Tanzania, followed by the evaluation of foliar-applied exogenous RNase A and boron on plant growth traits for MLND-infected plants, including plant height, stem diameter and aboveground biomass yield.

3.1 | Symptom Progression

Early symptom development was evident 2 days postinoculation, characterised by small, discrete leaf perforations arranged in a distinct pattern (Figure 3A). As the disease progressed, the holes enlarged, and faint linear yellowing became evident along the veins 5 days postinoculation (Figure 3B). At later stages (12 days postinoculation), severe chlorosis and necrosis developed (Figure 3C). Necrosis of the maize apical meristem was observed in 28.9% of the inoculated plants, leading to the death of the central shoot and the subsequent development of new lateral shoots (Figure 4). Figure 5 shows symptoms of MLND in inoculated maize plants and growth development of uninoculated maize plants before and after treatments. Table 2 summarises symptom progression

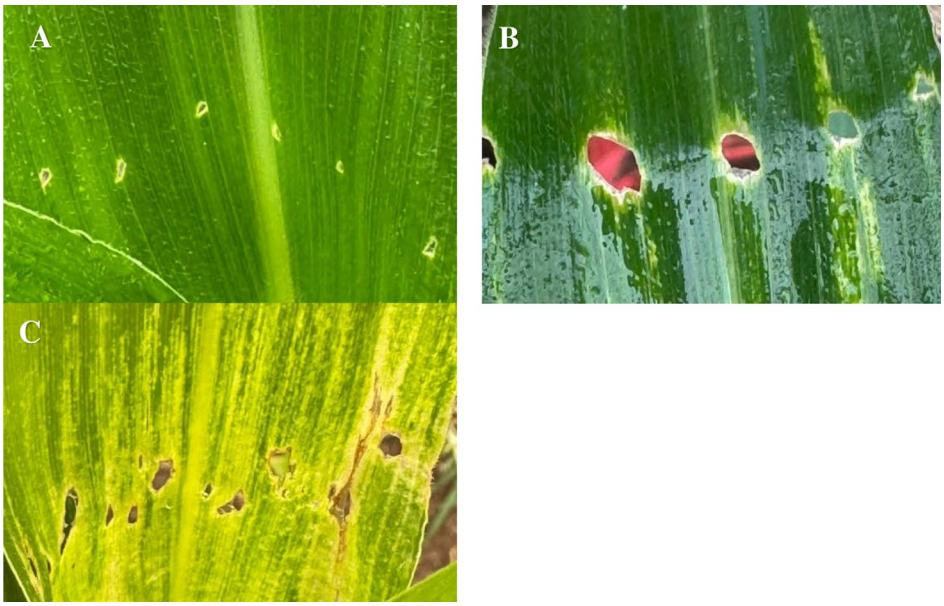

FIGURE 3 | Symptom development on leaves following suspected MLND inoculation. (A) Early symptoms show small, discrete holes forming in a distinct pattern on inoculated leaves (2 days postinoculation). (B) Progression of symptoms with enlargement of holes and appearance of faint yellowing along the veins (5 days postinoculation). (C) Advanced stage of infection, characterised by bright mosaic and the appearance of necrosis (12 days postinoculation).

observed in the stem-inoculated plants during the 21-day postinoculation observation period.

3.2 | Effects of Boron Treatments on Growth Parameters

The effect of the different boron concentrations on plant height, stem diameter and fresh and dry biomass yield is presented in Figure 6. Boron application enhanced vegetative growth in a concentration-dependent manner. B5, B10, and B15 treatments resulted in plant height of 41.1 ± 18.0 cm, 48.1 ± 13.5 cm, and 51.0 ± 18.2 cm, respectively (Figure 6A). The uninoculated control and the NT had a height of 57.3 ± 6.2 cm and 39.9 ± 16.4 cm, respectively. Statistical analyses (Tables S1 and S2) showed that plant height was statistically similar ($p > 0.05$) across the treatments. A similar trend was observed for stem diameter (Figure 6B). B15-treated plants resulted in a stem diameter of 1.08 ± 0.32 cm, followed by B10 (0.98 ± 0.38 cm) and B5 (0.92 ± 0.26 cm). The uninoculated control and NT recorded a stem diameter of 1.26 ± 0.20 cm and 0.84 ± 0.31 cm, respectively. The uninoculated control and NT were statistically different in stem diameter ($p = 0.040$); however, boron treatments (B5, B10, and B15) were statistically similar ($p > 0.05$) to the uninoculated control and NT. Figure 6C shows the effects of the treatments on aboveground fresh biomass yield. The uninoculated control, NT, B5, B10, and B15 treatments recorded a fresh shoot mass of 193.3 ± 49.0 g, 135.5 ± 27.4 g, 154.7 ± 49.4 g, 204.7 ± 48.6 g and 219.2 ± 68.0 g, respectively. Statistical analysis (Tables S1 and S2) shows a significant difference in NT/B10 ($p = 0.042$) and NT/B15 ($p = 0.009$) in fresh shoot mass. Results from the aboveground dry shoot mass show that the highest dry shoot mass was observed in B15 (72.4 ± 21.4 g), followed by B10 (67.3 ± 21.4 g) and B5 (45.1 ± 14.6 g) (Figure 6D). The uninoculated control and NT recorded a dry shoot mass of 64.0 ± 15.8 g and 44.3 ± 13.1 g, respectively. Statistical analyses (Tables S1 and S2) showed there were significant differences in dry shoot mass between NT/B15 ($p = 0.013$) and B5/B15 ($p = 0.017$).

3.3 | Effects of RNase A Treatment on Growth Parameters

The effects of the 14-day foliar exogenous RNase A application on plant height, stem diameter, fresh shoot mass and dry shoot mass are presented in Figure 7. The uninoculated control exhibited the highest plant height (57.3 ± 6.2 cm), stem diameter (1.26 ± 0.20 cm), fresh shoot mass (193.3 ± 49.0 g) and dry shoot mass (64.0 ± 15.8 g). This was followed by RNase12 with a plant height, stem diameter, fresh shoot mass and dry shoot mass of 41.5 ± 12.5 cm, 1.0 ± 0.3 cm, 141.3 ± 61.2 g and 46.1 ± 18.3 g, respectively. The lowest growth traits were observed in NT with plant height (39.9 ± 16.4 cm), stem diameter (0.84 ± 0.31 cm), fresh shoot mass (135.5 ± 27.4 g) and dry shoot mass (44.3 ± 13.1 g). Statistical analyses (Tables S3 and S4) showed a significant difference between the uninoculated control and NT in all the measured growth traits, such as height ($p = 0.018$), stem diameter ($p = 0.007$), fresh shoot mass ($p = 0.044$) and dry shoot mass ($p = 0.037$), and a significant difference between the uninoculated control and RNase12 in plant height ($p = 0.032$). The RNase12 treatments on plant height, stem diameter, fresh shoot mass and dry shoot mass were statistically similar ($p > 0.05$) to NT.

3.4 | Molecular and Serological Results

Results from PCR-based detection of MCMV and SCMV (Figure 8A,B) showed that the field samples used for inoculation

FIGURE 4 | Symptoms associated with apical necrosis and side shoot formation in a suspected MLND-infected maize plant. (A) Maize plant showing symptoms of necrotic spots on the apical meristem (7–10 days postinoculation). (B) Maize plant showing necrosis (death) of the apical meristem, resulting in loss of the central shoot in inoculated plants (10–14 days postinoculation). (C) Close-up view of the necrotic (dead) apical meristem. (D) Same plant showing subsequent development of new lateral shoots (12–15 days postinoculation).

tested positive for both MCMV and SCMV. After treatment, MCMV was detected in leaf tissues from the NT treatment, B5 and B10 treatments, whereas the uninoculated control and the B15 treatment tested negative for MCMV. After treatment, SCMV was not detected in leaf tissues from any treatment except in one of the field samples used for inoculation. Results from MCMV AgriStrips show a positive detection of MCMV in the field sample used in the inoculation, NT, B5 and B10 after treatments, and no detection in the uninoculated control and the B15 after treatment (Figure 9).

4 | Discussion

This study evaluated the effects of boron and exogenous RNase A on growth performance and symptom development in MLND-infected maize plants. The results showed that MLND infection significantly reduced plant growth, while boron application improved biomass yield. In contrast, RNase A treatment did not result in significant improvements in growth parameters. Symptom progression followed a distinct pattern consistent with MLND infection.

Key symptoms observed in the MLND-infected maize plants in this study included mosaic mottling, chlorosis, necrosis, apical meristem death and lateral shoot development. These symptoms are consistent with previous reports of MLND progression (Nelson et al. 2011; Suresh 2019; Terefe and Gudero 2019; Uyemoto et al. 1981; Zhan et al. 2022). In some plants, the disease extended to the growing point, causing apical meristem death (dead heart symptom) and complete growth arrest. This observation agrees with a previous report on MCMV and MLND in Kenya, which shows that necrosis of young leaves led to a 'dead heart' symptom (Wangai et al. 2012). The absence of these symptoms in uninoculated plants confirms that the observed responses were associated with MLND infection rather than environmental or nutritional factors.

Symptom development followed a clear temporal progression, beginning with small, discrete leaf perforations at 2 days postinoculation, followed by chlorotic streaking and lesion expansion, and culminating in severe chlorosis, necrosis, and in some cases, apical meristem death. This progression reflects systemic viral movement within the plant. The use of stem inoculation in this study may have facilitated rapid systemic spread by providing direct access to the vascular system. This is consistent with previous studies showing that plant viruses move systemically through vascular tissues following initial infection (Dolja et al. 1992; Rodrigo et al. 2014), and that bypassing early localised infection stages can accelerate symptom development (Zhao et al. 2025; Zwart et al. 2012).

FIGURE 5 | **Upper panel**: Representative images of experimental plants (A) uninoculated plant (control) and (B—F) inoculated plants before treatment. **Middle panel**: Representative images of (G) uninoculated plant with no treatment, (H) inoculated plant with no treatment, (I) inoculated plant with RNase A treatment (12 μg mL^{-1}). (J) inoculated plant with boron treatment at 5 mg L^{-1}, (K) inoculated plant with boron treatment at 10 mg L^{-1}, and (L) inoculated plant with boron treatment at 15 mg L^{-1}, 14 days after treatment. **Lower panel**: Close-up view of the apical meristem of (M) uninoculated plant (control), (N) inoculated plant with no treatment, (O) inoculated plant with RNase A treatment (12 μg mL^{-1}), (P) inoculated plant with boron treatment at 5 mg L^{-1}, (Q) inoculated plant with boron treatment at 10 mg L^{-1}, and (R) inoculated plant with boron treatment at 15 mg L^{-1}.

TABLE 2 | Symptomatology of MLND maize plants during the 21-day postinoculation period.

Symptom category	Specific symptom	Description	Time of first observation
Leaf symptoms	Leaf perforation	Small discrete holes forming in a distinct pattern	2 dpi
	Progressive lesion development	Enlargement of holes accompanied by faint yellowing along leaf veins	5 dpi
	Chlorosis	Yellowing along leaf margins and veins, intensifying toward the midrib.	3–5 dpi
	Mosaic pattern	Bright distinct mosaic characteristic	6–9 dpi
	Necrotic lesions	Appearance of necrotic spots and localised tissue death.	7–12 dpi
Apical meristem symptoms	Necrotic spots	Visible brown spots around apical meristematic region.	7–10 dpi
	Apical necrosis	Death of the apical meristem leading to loss of the central shoot.	10–14 dpi
	Side shoot formation	Development of lateral shoots due to damage/loss of apical meristem.	12–15 dpi

Note: dpi = days post inoculation.

In advanced stages of infection, additional symptoms associated with apical necrosis and compensatory shoot development were observed in the MLND-infected maize plants. Some inoculated plants exhibited complete death of the apical meristem, resulting in the loss of the central growing point and the arrest of vertical growth. Following apical death, the same plants initiated the

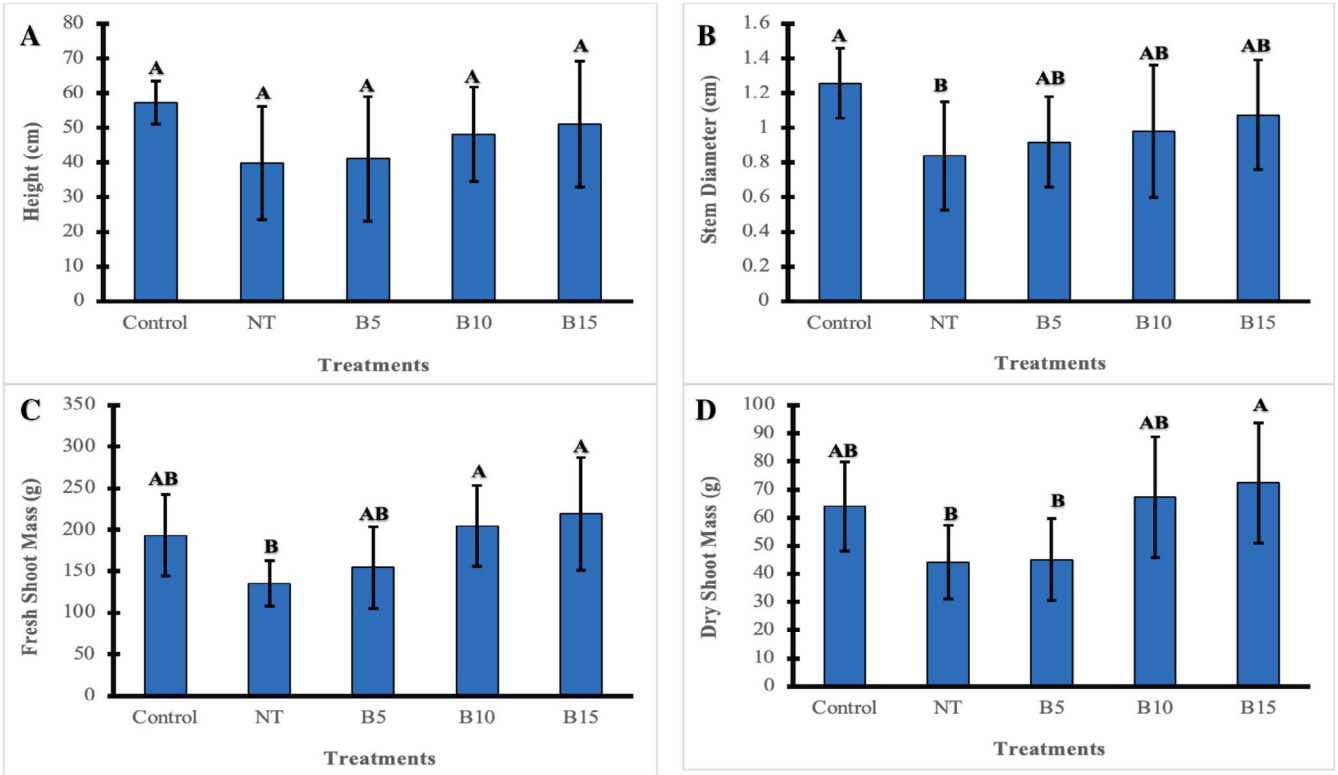

FIGURE 6 | Effect of boron treatments on (A) height, (B) stem diameter, (C) fresh shoot mass, and (D) dry shoot mass. Data represent mean values and standard deviation ($n = 9$) of three replications. Bars with different letters are significantly different ($p < 0.05$). Control (uninoculated plant with no treatment), NT (inoculated plant with no treatment), B5 (inoculated plant with boron treatment at 5 mg L^{-1}), B10 (inoculated plants treated with boron at 10 mg L^{-1}), and B15 (inoculated plants treated with boron at 15 mg L^{-1}).

development of new lateral shoots from the lower nodes, consistent with compensatory growth through activation of axillary meristems. This mechanism of side shoot formation reflects the plant's attempt to restore vegetative growth. This observation is consistent with a previous study that reported that apical dominance, as a central mechanism, suppresses the growth of the axillary bud through the apical meristem growth (McSteen and Leyser 2005). When the apical meristem is removed, this suppression is released, enabling the outgrowth of the lateral bud (Beveridge 2006).

The symptom progression documented in this study has practical relevance for disease identification in regions where access to molecular diagnostic tools is limited. In many smallholder farming systems in SSA, routine laboratory testing is often not feasible due to cost and infrastructure constraints (Klauser 2018; Miller et al. 2009). The clear temporal sequence of symptoms observed in this study provides a useful framework for field-based identification of MLND. Such symptom-based identification can support early detection and timely management decisions in resource-limited settings.

MLND is known to cause severe plant stunting, as reported by previous studies (Mahuku et al. 2015; Uyemoto et al. 1981). Results from this study in the exogenous RNase A treatment show significant differences in plant height between the inoculated plants with no treatment and the uninoculated control group. Similarly, plants treated with exogenous RNase A did not differ significantly from the inoculated, untreated group, indicating that exogenous RNase A application did not mitigate MLND-associated growth suppression. In contrast, boron-treated plants exhibited plant heights that were not significantly different from the uninoculated control. This suggests that boron application may have mitigated the stunting effect associated with MLND to a level comparable to healthy plants under the conditions tested. Similar results were observed for stem diameter, and fresh and dry aboveground biomass yield between the inoculated plants with no treatment and the uninoculated control group. This finding confirms the strong negative impact of MLND on maize vegetative growth.

Foliar application of exogenous RNase A at 12 $\mu g\,mL^{-1}$ did not result in a significant improvement in the growth traits (stem diameter, fresh and dry aboveground biomass yield) of the plant, with RNase A-treated plants showing no statistically significant differences ($p > 0.05$) relative to either the uninoculated control or the inoculated, untreated group. The lack of differentiation from the inoculated, untreated group highlights the need for further optimisation of application rate or mode of application to determine its potential role in MLND management. An increase in boron concentration (15 mg L^{-1}) increased the aboveground biomass yield of the MLND-infected maize plants significantly ($p < 0.05$) compared to the inoculated plant with no treatment. This observation agrees with previous studies, which indicate an increase in plant biomass yield with boron application (Franco-Lagos et al. 2023; Saleem et al. 2020; Tahir et al. 2012; Tayyab et al. 2022). According to Tahir et al. (2012), the increase in growth traits of maize plants following foliar boron

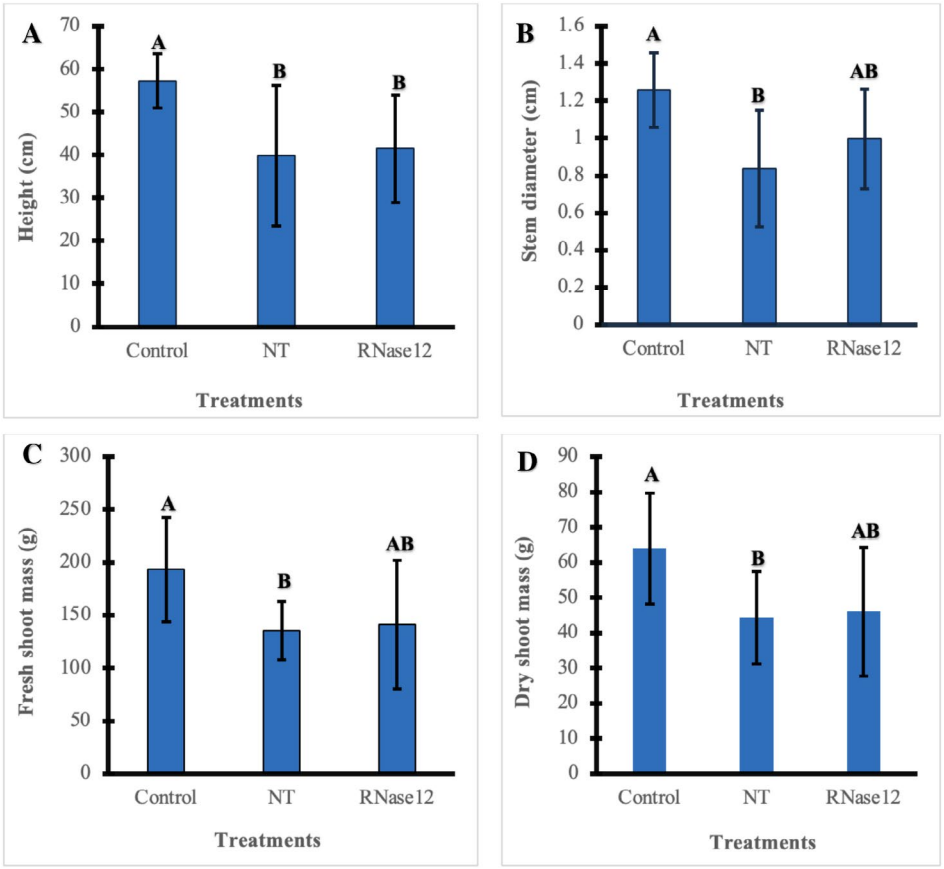

FIGURE 7 | Effect of exogenous RNase A treatment on (A) height, (B) stem diameter, (C) fresh shoot mass, and (D) dry shoot mass. Data represent mean values and standard deviation ($n = 9$) of three replications. Bars with different letters are significantly different ($p < 0.05$). Control (uninoculated plant with no treatment), NT (inoculated plant with no treatment), and RNase12 (inoculated plants treated with exogenous RNase A at 12 ug L^{-1}).

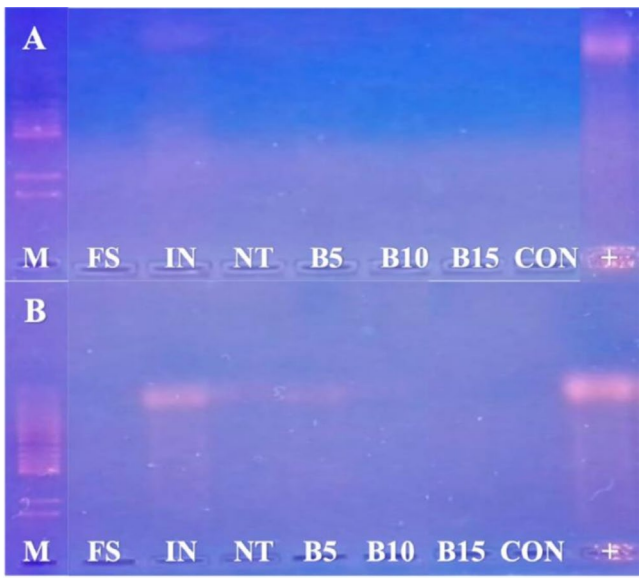

FIGURE 8 | PCR amplification for (A) sugarcane mosaic virus (SCMV) and (B) maize chlorotic mottle virus (MCMV) of FS (Field sample not showing symptoms of maize lethal necrosis disease); IN (Field sample showing symptoms of maize lethal necrosis disease used as an inoculum); NT (inoculated group with no treatment); B5 (inoculated group with boron treatment at 5 mg L^{-1}); B10 (inoculated group with boron treatment at 10 mg L^{-1}); B15 (inoculated group with boron treatment at 15 mg L^{-1}); CON (uninoculated control group) after treatment.

application may be due to its key role in cell wall synthesis, division, elongation and nucleic acid metabolism. The significant increase in biomass yield of the MLND-infected maize plants with increasing boron application observed in this study may be attributed to boron's role in sustaining meristematic activity and supporting vascular development under MLND-associated physiological stress.

The molecular and serological results provide mechanistic support for these growth responses. PCR analysis revealed the presence of MCMV in field samples used for the inoculation, and in the NT treatment, as well as in the B5 and B10 treatments, whereas MCMV was not detected in the uninoculated control or the B15 treatment after treatment. Further confirmation using MCMV AgriStrips aligned with the PCR findings, with positive detection of MCMV only in the field samples used for the inoculation, NT, B5 and B10 treatments. The consistent absence of detectable MCMV in B15-treated plants suggests that boron application at 15 mg L^{-1} effectively suppressed viral accumulation to below detectable levels. Although SCMV was detected in the field sample used for the inoculation, it was not detected in leaf tissues following treatment. This may reflect lower SCMV titres in the various treatments. Given the central role of MCMV in MLND development, the elimination of detectable MCMV in B15-treated plants likely explains the observed recovery in biomass production relative to inoculated, untreated plants. Although boron has been investigated for potential inhibitory

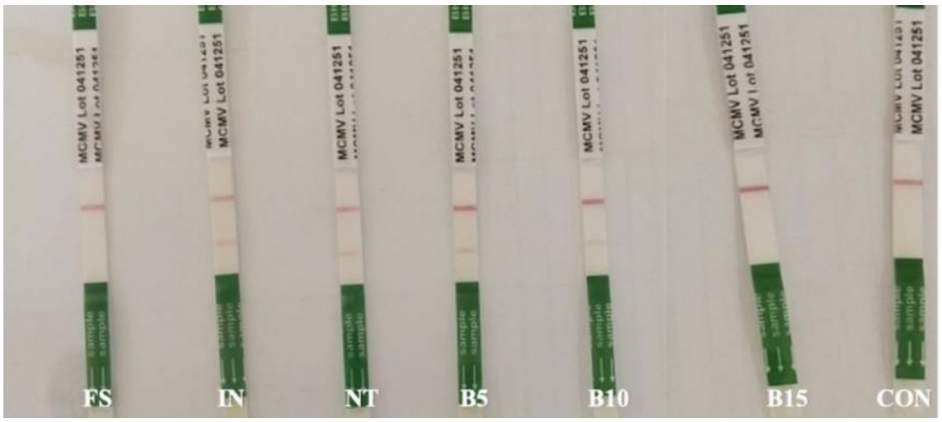

FIGURE 9 | Maize chlorotic mottle virus (MCMV) detection test using AgriStrip for FS (Field sample not showing signs of maize lethal necrosis disease); IN (Field sample showing symptoms of maize lethal necrosis disease used as an inoculum); NT (inoculated group with no treatment); B5 (inoculated group with boron treatment at 5 mg L^{-1}); B10 (inoculated group with boron treatment at 10 mg L^{-1}); B15 (inoculated group with boron treatment at 15 mg L^{-1}); and CON (uninoculated control group) after treatment.

effects on phytopathogens, such mechanistic studies have been limited to only a few pathosystems. One previous report showed that high concentrations of boron inhibited glycolysis, thereby reducing the growth of two fungal species, *Saccharomyces cerevisiae* and *Penicillium chrysogenum* (Bowen and Gauch 1966). The exact mechanism by which boron suppresses MCMV accumulation and enhances biomass yield remains unclear, and future studies should investigate the underlying physiological and molecular pathways.

Phenotypic, molecular and serological evidence indicate a dose-dependent effect of boron, with the 15 mg L^{-1} concentration conferring both improved growth performance and suppression of MCMV accumulation. These findings highlight the potential role of optimised boron nutrition as a complementary component of integrated MLND management strategies.

5 | Limitations and Future Studies

One limitation of this study is the absence of molecular and serological analyses for the RNase A (12 μg mL^{-1}) treatment. Because RNase A-treated plants showed no significant improvement in growth traits relative to the inoculated, untreated group and continued to exhibit visible mosaic mottling symptoms after treatment, viral detection assays were not pursued. A recent study demonstrated that the addition of RNase A to hop latent viroid (HLVd)-infected sap did not degrade the viroid RNA when compared to in vitro studies involving purified RNA, suggesting the presence of inhibitors in the plant sap (Punja et al. 2025). A similar result was observed in our unpublished data, which show that the application of exogenous RNase A degraded HLVd RNA in hydroponic nutrient solution but not in the roots of the plants.

Symptom development was assessed based on the timing of symptom onset rather than by quantitative disease severity ratings, which limits the ability to compare disease progression across treatments. Growth measurements were recorded at two time points (7 and 21 days postinoculation); however, intermediate growth-stage assessments were not included, which limits insight into temporal responses to treatments. The study was conducted using a single maize cultivar under screenhouse conditions, and grain yield was not measured, which may limit the generalisability and direct agronomic applicability of the findings under field conditions.

Future studies should incorporate standardised disease severity indices to strengthen the evaluation of treatment effects on disease progression. Finally, field-based trials should include grain yield assessment to validate the practical relevance and scalability of these findings for smallholder farming systems.

6 | Conclusion

This study set out to monitor and characterise the early development and progression of symptoms associated with MLND in Tanzania, and to evaluate the effects of exogenous RNase A and boron application on the growth performance of the MLND-infected maize plants. The symptomology documented in this study, ranging from early perforations and streaking to systemic chlorosis, necrosis and apical meristem death, provides a detailed profile of disease development that may strengthen symptom-based detection frameworks in regions where access to molecular diagnostics is limited. Such visual diagnostic tools are essential for rapid and low-cost disease surveillance in smallholder-dominated agricultural systems across SSA.

Foliar application of exogenous RNase A at 12 μg mL^{-1} did not significantly ($p > 0.05$) improve growth traits of MLND-infected maize plants compared with the inoculated, untreated controls; however, foliar boron application at 15 mg L^{-1} effectively suppressed MCMV in the infected plants and resulted in a significantly higher aboveground fresh biomass yield ($p = 0.009$) and aboveground dry biomass yield ($p = 0.013$) compared with the inoculated plant with no treatment, highlighting the potential role of micronutrient management in reducing MLND-related physiological stress. More targeted research is needed to understand the physiological or biochemical pathways behind this response. Overall, these findings enhance our understanding of MLND symptom development and provide essential evidence to

support the development of affordable, field-friendly strategies to decrease disease severity and boost maize yields in Tanzania and the broader SSA region.

Author Contributions

P.Y.K.: conceptualisation, formal analysis, investigation, methodology, software, writing – original draft, writing – review and editing. O.A.: writing – review and editing. P.W.A.: formal analysis, writing – review and editing. S.M.: writing – review and editing, formal analysis. P.M.: resources, supervision, writing – review and editing. M.L.: conceptualisation, funding acquisition, resources, supervision, writing – review and editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

Peer Review

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Table S1:** Statistical analysis of boron treatments for suspected MLND-infected maize plant growth parameters. **Table S2:**. Statistical analysis (*p* value) of the interaction of the boron treatments on the growth traits of the suspected MLND-infected maize plant using the Tukey–Kramer test. **Table S3:** Statistical analysis of exogenous RNase A treatment for suspected MLND-infected maize plant growth parameters. **Table S4:** Statistical analysis (*p* value) of the interaction of the various treatments on the growth traits of the suspected MLND-infected maize plant using the Tukey–Kramer test.