

*Full Length Research Paper*

# Screening of maize germplasm for resistance to maize lethal necrosis disease in Zambia

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**Maize is an important staple crop for humans and livestock feed in Zambia. A total of 473 maize germplasms were screened under maize lethal necrosis (MLN) artificial inoculation and disease pressure using an alpha lattice design to identify potential tolerant varieties as sources of resistance. The analysis of variance (ANOVA) based on the MLN score range of 1-9 showed that the levels of MLN resistance in the maize germplasms were very low, with almost all the hybrids widely grown in Zambia and germplasms drawn from the genebank found to be highly susceptible. However, the pro vitamin A varieties, GV662A and IICZ3085 showed a moderate level of resistance/tolerance, with disease scores of 4.7 and 5.3, respectively. There is need to enhance development of MLN tolerant maize cultivars by the national maize breeding programme and seed companies to avert the possible calamity posed by the threat of the effects of MLN. In addition, intensive awareness creation among various stakeholders in the maize value chain, systematic monitoring and surveillance of MLN, practicing integrated disease management approaches such as avoiding maize monoculture and continuous cultivations of susceptible maize throughout the year, and practicing maize crop rotation with compatible crops especially legumes that do not serve as hosts for maize chlorotic mottle virus (MCMV)/MLN are recommended.**

**Key words:** Maize lethal necrosis, screening, maize germplasm, Zambia.

## INTRODUCTION

Maize is an important staple crop for humans and livestock feed accounting for 60% of national calorie consumption and serves as the dietary mainstay in Zambia (Dorosh et al., 2009). The crop has a consumption level of over 130 kg per capita annually

(Shiferaw et al., 2011). It is grown throughout the country of which about 80% produced by the majority small holder farmers is estimated at 2,394,907 metric tonnes and cultivated on an area of 1,086,000 ha with average yields of 2.2 t/ha (FAOSTAT, 2018). According to Kassie

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et al. (2012), most of the cultivated maize area is covered with hybrid maize (65.49%) followed by local varieties (28.5%) and improved open-pollinated varieties (6.0%). The maize seed industry in Zambia is highly developed and regarded as one of the major commercial maize-seed producer in southern Africa (Boddupalli et al., 2020).

The increasing global spread of transboundary pests and diseases poses a threat to the food security, income and livelihood of numerous resource-poor farmers particularly in southern Africa whose 85% of the maize produced is for food consumption (Boddupalli et al., 2020). The occurrence and outbreak of maize lethal necrosis (MLN), first reported in Kenya (Wangai et al., 2012) and later on in Rwanda (Adams et al., 2014), D.R. Congo (Lukanda et al., 2014), Taiwan (Deng et al., 2014), Uganda (Boddupalli et al., 2020), Ethiopia (Mahuku et al., 2015a), Ecuador (Quito-Avila et al., 2016) and Spain (Achon et al., 2017), stand as the major current risk and danger to the maize and seed production efforts in southern Africa (Boddupalli et al., 2020). The cause of MLN is as a result of the synergistic co-infection of maize by *maize chlorotic mottle virus* (MCMV; genus *Machlomovirus*, family Tombusviridae) and with any one of the viruses from the family Potyviridae, such as *sugarcane mosaic virus* (SCMV), *maize dwarf mosaic virus* (MDMV) or *wheat streak mosaic virus* (WSMV) (Uyemoto et al., 1980; Redinbaugh and Stewart, 2018). Coupled with abiotic stresses, a number of other potyviruses can similarly cause synergistic reactions in co-infections with MCMV and can aggravate MCMV infection to cause MLN (Redinbaugh and Zambrano-Mendoza, 2014). While the maize-infecting potyviruses are generally common; the outbreak of MLN in Africa has been driven by the emergence and spread of MCMV (Mahuku et al., 2015b). There is experimental evidence pointing to the possibility of thrips and other vectors to be playing a major role in MCMV movement, thus MLN, within and between fields in the affected countries in Africa (Mahuku et al., 2015b). The MCMV can also be transmitted by seed contamination, a mechanism which can contribute to speedy and long-range spreading of the MLN disease (Jensen et al., 1991; Zhang et al., 2011).

The impact of MLN on maize production in eastern Africa has been reported (De Groote et al., 2016; Marenya et al., 2018) with estimated maize yield losses ranging between 23 and 100% in Kenya (Boddupalli et al., 2020). The disease remains a key hazard to the maize crops in eastern Africa (Isabirye and Rwomushana, 2016), and therefore, poses as the biggest threat to its emergence in other sub regions especially southern Africa (Boddupalli et al., 2020). The recent survey data in the southern Africa shows the sustained absence of MCMV/MLN in Tanzania, Malawi, Zambia and Zimbabwe (Boddupalli et al., 2020). Zambia Agriculture Research Institute (ZARI) has been developing maize varieties tolerant to biotic stresses.

However, breeding for MLN was not included in the breeding programme since the disease does not occur in the country. The outbreak of MLN in east and central Africa has brought up measures to curb the spread of the disease by restricting germplasm exchange, seed trade, grain export or imports (Boddupalli et al., 2020).

This study evaluated the response of maize varieties from the private and public seed institutions as well as maize accessions from the National Plant Genetic Resources Centre (NPGRC) of Zambia to MLN. The results would guide the maize breeding programs in Zambia to identify germplasm that is tolerant and/or resistant to MLN and help to prepare and respond to possible outbreak of the disease in Zambia.

## MATERIALS AND METHODS

### Assembly of maize varieties and genebank accessions

The establishment of the KALRO \_ CIMMYT MLN Screening Facility at the KALRO-Naivasha Dairy Research Center, Kenya in September 2013 has allowed for screening of a large number of maize germplasms against MLN under artificial inoculation from different countries (Boddupalli et al., 2020). For this study, a total of 473 maize germplasms (Table 1) were collected from the major seed companies, the national maize breeding team and the NPGRC and were sent to Naivasha, Kenya for MLN field evaluation. The collected maize germplasms comprised accessions and landraces from the NPGRC, hybrids and open pollinated varieties released in Zambia commonly cultivated from seed companies (Seedco, Zamseed, Pioneer, Pannar) and ZARI.

### Trial design, artificial inoculation and disease scoring

The trials were established under MLN artificial inoculation at Naivasha using an alpha lattice design. The entries were planted in two-row plots, 3 m long, with rows spaced at 0.75 m between rows. Two seeds per station were planted at 0.25 m intervals and the stands were thinned to one plant per station 3 weeks after emergence to obtain a final plant population density of 53,333 plants per hectare. All recommended agronomic management practices such as land preparation, weeding, fertilization and pest control were followed.

The maintenance of MCMV and sugarcane mosaic virus (SCMV) in susceptible host plants and preparation of MLN inoculum for artificial inoculation was done using the optimized protocols as described by Gowda et al. (2015) and Sitonik et al. (2019). The inoculum for the MLN field trial was prepared by following an optimized combination of the SCMV and MCMV viruses (ratio of 4:1). The infected leaves, verified prior by the Enzyme-Linked Immunosorbent Assay (ELISA), were weighed, chopped and homogenized in 0.1 M potassium phosphate buffer in a 1:10 dilution at pH 7.0. The inoculum was sieved through a nylon mesh paint strainer and 0.02 g/ml of Carborundum was added. The MLN inoculum was applied to the maize seedlings mechanically by using a motorized, back-pack mist blower (Solo 423 Mist Blower, 12 L capacity). An open-nozzle (2-in. diameter) was used to deliver inoculum spray at a pressure of 10 kg/cm<sup>2</sup>. To reduce any possibility of escapes and ensure uniform inoculation, the inoculation on the maize seedlings was carried out twice at one-week intervals with the first at 4-6 leaf stage and a second 7 days after the first inoculation. Across all entries, any symptomatic plants observed before inoculation were discarded. The presence of both

**Table 1.** Maize germplasms used in this study

Group	Germplasm	Source	Remarks	Reference(s)
1	ZM 4623, ZM 4380, ZM 4631, ZM 5191, ZM 4429, ZM 4342, NPGRC 4267, ZM 6815, ZM 8173, ZM 5017, ZM 5698, ZM 4444, NPGRC 4311, ZM 7035, ZM 4451, ZM 4440, NPGRC 4352, ZM 4764, ZM 7353, ZM 8224, ZM 4760, ZM 4455, ZM 8181, ZM 4618, ZM 4418, ZM 4763, ZM 4632, ZM 8231, ZM 4414, ZM 4419, ZM 4417, ZM 4748, ZM 4369, ZM 8259, ZM 5649, ZM 4753, ZM 4416, ZM 8265, NPGRC 4361, ZM 4625, ZM 4630, ZM 5173, ZM 8115, ZM 5645, ZM 8172, ZM 4249, NPGRC 4363, ZM 8235, ZM 5385, ZM 5387, ZM 4368, ZM 5190, ZM 4616, ZM 4384, ZM 4453, ZM 4629, ZM 4367, ZM 4352, ZM 5034, ZM 5196, ZM 4431, ZM 5490, ZM 4764, ZM 4610, ZM 5462, ZM 4381, ZM 8180, ZM 4773, ZM 4342, ZM 4432, ZM 8298, ZM 5210, ZM 4735, ZM 2375, ZM 4762, ZM 4307, ZM 4359, ZM 5631, ZM 4348, ZM 8211, ZM 4423, ZM 4736, ZM 4754, ZM 4347, ZM 8232, ZM 6593, ZM 4350, ZM 5189, ZM 4733, ZM 5043, ZM 8239, ZM 4613, ZM 4433, ZM 4313, ZM 4413, ZM 4772, ZM 4413, ZM 4339, ZM 4756, ZM 4415, ZM 4041, ZM 4375, ZM 4749, ZM 4266, ZM 5200, MMV 405, MMV 415, MMV 420, MMV 530, POP10, POP25, ZM421, ZM521, ZM621, OBATANPA, MMV 409	ZARI	ZARI Maize accessions and ZARI released varieties	NPGRC; Badu-Apraku et al. (2006)
2	ZM 4262, NPGRC 4309, ZM 6777, ZM 4369, NPGRC 4269, ZM 4236, ZM 4617, ZM 4373, ZM 6863, ZM 5207, ZM 5686, ZM 8208, ZM 4367, ZM 4238, ZM 4422, ZM 4456, ZM 5021, ZM 4619, ZM 4239, ZM 6593, ZM 4366, ZM 8188, ZM 8162, ZM 4261, ZM 5191, ZM 4747, ZM 5667, ZM 4442, ZM 4439, ZM 4622, ZM 4376, ZM 4327, ZM 4626, ZM 4264, ZM 4314, ZM 6967, ZM 4458, ZM 4374, ZM 5209, ZM 4430, ZM 4628, ZM 4382, ZM 4378, ZM 5020, ZM 5383, ZM 7418, ZM 4255, ZM 4612, ZM 7148, ZM 4611, ZM 4263, ZM 8252, ZM 4448, ZM 4370, ZM 5705, ZM 4240, NPGRC 4318, ZM 5207, ZM 4454, ZM 6964, ZM 4237, ZM 4627, ZM 6017, ZM 5047, ZM 4308, ZM 4347, ZM 4436, ZM 8243, ZM 4424, ZM 7010, ZM 4240, ZM 4768, ZM 4744, ZM 5195, ZM 5643, ZM 4739, ZM 4360, ZM 4447, ZM 6984, ZM 4766, ZM 4624, ZM 5666, ZM 4735, ZM 4376, ZM 6981, ZM 4428, ZM 4622, ZM 4457, ZM 5208, ZM 4450, ZM 4761, NPGRC 4356, ZM 4613, ZM 4447, ZM 4245, ZM 4315, ZM 4605	ZARI	ZARI Maize accessions	NPGRC
3	ZM 7120, ZM 7397, ZM 7285, ZM 8262, ZM 8238, ZM 4256, ZM 4335, ZM 8236, ZM 3641, ZM 7396, ZM 7435, ZM 5196, ZM 4253, ZM 6653, ZM 7476, ZM 8219, ZM 4329, ZM 7456, ZM 8213, ZM 8254, ZM 4325, ZM 5197, ZM 5039, ZM 7285, ZM 4254, ZM 7235, ZM 4268, ZM 8214, ZM 4259, ZM 6860, ZM 4383, ZM 6656, ZM 8253, ZM 8226, ZM 7380, ZM 7373, ZM 4242, ZM 8215, ZM 8256, ZM 8183, ZM 7267, ZM 4358, ZM 4233, ZM 8195, ZM 8190, ZM 6639, ZM 7324, ZM 5194, ZM 4757, ZM 4385, ZM 8219, ZM 4758, ZM 7355, ZM 4234, ZM 4745, ZM 4232, ZM 7374, ZM 8171, ZM 8184, ZM 4750, ZM 8207, ZM 6706, ZM 7145, ZM 4353, ZM 5708, ZM 4622, ZM 8201, ZM 4425, ZM 6594, ZM 7146, ZM 4771, ZM 5680, ZM 4321, ZM 4257, ZM 6846, ZM 4609, NPGRC 4260, ZM 4420, ZM 4434, ZM 7019, ZM 8230, ZM 4365, ZM 6785, ZM 4614, ZM 4615, ZM 4235, ZM 5675, ZM 7275, ZM 4737, ZM 4606, ZM 4344, ZM 4438, ZM 8163, ZM 4334, ZM 5697, ZM 5710, ZM 4765, ZM 6957, ZM 4452	ZARI	ZARI Maize accessions	NPGRC
4	ZM 8248, ZM 7236, ZM 4426, ZM 4755, ZM 8185, ZM 4748, ZM 7421, ZM 4742, ZM 8187, ZM 4246, ZM 4752, ZM 7002, ZM 7441, ZM 7422, ZM 7120, ZM 5214, ZM 7153, ZM 7141, ZM 8228, ZM 8192, ZM 7151, ZM 8251, ZM 7348, ZM 5215, ZM 4437, ZM 7336, ZM 7147, ZM 5205, ZM 7237, ZM 8157, ZM 5213, ZM 7007, ZM 7254, ZM 6639, ZM 8225, ZM 4250, ZM 5045, ZM 5216, ZM 7038, ZM 6815, ZM 7171, ZM 7446, ZM 4768, ZM 4429, ZM 6968, ZM 7233, ZM 8237, ZM 8157, ZM4756, ZM 7142, ZM 7305, ZM 5642, ZM 4445, ZM 5984, ZM 7318, ZM 6965, ZM 4607, ZM 4741, ZM 7305, ZM 6843, ZM 8197, ZM 7427, ZM 8260, ZM 7263, ZM 8244, ZM 7246, ZM 4421, ZM 4323, ZM 6843, ZM 7315, ZM 6790, ZM 4746, ZM 6623, ZM 6611, ZM 5194, ZM 6846, ZM 4745, ZM 8223, ZM 5203, ZM 6866, ZM 4771, ZM 5210, ZM 4734, ZM 8196, ZM 5210, ZM 7143, ZM 4335, ZM 8174, ZM 7433, ZM 7012, ZM 5198, ZM 5042, ZM 6628, ZM 8165, ZM 7354, ZM 4251, ZM 7114, ZM 8220	ZARI	ZARI Maize accession	NPGRC
5	MM 441, ZMS 405, ZMS 528, ZMS 606, ZMS 616, ZMS 620, ZMS 623, ZMS 638, ZMS 652, ZMS 720, ZMS 721, ZMS 722, ZMS 607Y, ACCROSS 917, DKC 8053, DKC 8033, DKC 9053, DKC 8031, DKC 9089, DKC 8073, P2859W, PHB 30G-19, P3812W, PHB 3253, PAN 8M-93 (LOT #: 1412-ZEM-27366) - R2, PAN 12 (LOT #: N/174504/DAD) - F3, PAN 8M-91 (LOT #: 14911-ZEM-20204) - R3, PAN 413 (LOT #: M/199904/JAD) - F3, PAN 6777 (LOT #: 14911-ZEM-20297) - F3, PAN 4M-21 (LOT #: 13911-ZEM-20876B) - F3, PAN 7M-83 (LOT #: N/198504/IAD) - R4, PAN 4M-19 (LOT #: N/177304/EAD) - F3, PAN 6227 (LOT #: K/575401/DAA) - F3-60K, PAN 14 (LOT #: M/144903/FAC) - F3, PAN 53 (LOT #: 14911-ZEM-20266) - R2, PAN 7M-81 (LOT #: N/194404/HAD) - R2-50K, PAN 69 (LOT #: K/260901/IAA) - SFS, 14CZ405, SC 647, SC 637, SC 627, SC 719, 14CZ404, SC 727, 11CZ3087, 11CZ3085, MRI 734, MRI 514, MRI 614, MRI 624, MRI 744, MRI 594, MRI 634, MRI 724, ZM 421, ZM 521, ZM 621, OBATANPA, POP10, POP25, GV 662A, GV 664A, GV 665A	ZAMSEED, Monsanto, MRI-Syngenta, Pannar Seed, Pioneer, ZARI	Popular maize hybrids in Zambia, ZARI Maize varieties	Badu-Apraku et al. (2006), Masole and Gumbo (1994), Howard and Mungoma (1996), Smale et al. (2013), and Mubanga et al. (2014; 2018)

NPGRC-National Plant Genetic Resources Centre (<http://www.fao.org/pgra-gpa-archive/zmb/nfp.html>)/Zambia; ZARI-Zambia Agricultural Research Institute; MRI-Syngenta Maize Research Institute-Syngenta seed company.

**Table 2.** Analysis of variance and F-statistic tests for stand count (SC) and 10-days interval four MLN severity scores when assessing 473 maize germplasms.

Source of variation	Df	Measured parameters					
		SC	MLN 1	MLN 2	MLN 3	MLN 4	AUDPC
Germplasm	435	2289.7	74.4	41.4	92.4	90.8	34859.1
Residual	37	131.5	6.8	2.8	3.2	2.6	1492.2
Total	472	2421.2	81.2	44.2	95.7	93.4	36351.3
Mean		12.4	5.5	6.1	6.6	7.5	191.6
CV %		15.2	7.8	4.5	4.5	3.5	3.3
<i>P</i>		0.072	0.6	0.2	0.001	0.001	0.006
Significance		ns	ns	ns	***	***	***

ns = non-significant; \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.001$ , Df = degrees of freedom, SC = stand count; MLN 1 to MLN 4 denote Maize Lethal Necrosis severity score (1-9) recorded at 10, 20, 30 and 40 days after the second inoculation; AUDPC = Area under disease progress curve; CV = Coefficient of variation;  $p$  = F probability test.

viruses (MCMV and SCMV) in the field trial was confirmed by ELISA. The MLN disease severity (DS) was visually scored on each plot in an ordinal scale of 1 (completely clean plants with no visible MLN disease symptoms); 2 (fine or no chlorotic specks, but no loss of plant vigor); 3 (mild chlorotic streaks on emerging leaves); 4 (moderate chlorotic streaks on emerging leaves); 5 (chlorotic streaks and mottling throughout the plant); 6 (intense chlorotic mottling throughout the plant, with necrosis of leaf margins); 7 (severe chlorotic mottling, mosaic, and leaf necrosis all through the plant); 8 (severe chlorotic mottling, leaf necrosis, dead heart and premature death of plants) and 9 (complete plant necrosis, and dead plants) (Boddupalli et al., 2020).

In order to ensure that MLN was determined at the low, medium and high levels in all germplasm, data were recorded at 10-day intervals, beginning from 14 days after the second inoculation for up to four observations. For the DS analyses, after analyzing each time score, a third score was used (40 days post-inoculation) which also had high heritability compared to other scores. The analysis of variance for each trial was performed using Genstat for Windows 20th Edition, VSN International (2019). The area under the disease progress curve (AUDPC) was calculated for each plot to provide a measure of the progression of MLN severity across time using the method described by Jones et al. (2007).

## RESULTS

### Analysis of variance (ANOVA) for stand count, maize lethal necrosis severity and area under disease progress curve (AUDPC)

The average plant stand count was 12.4 with the lowest and highest being 1 and 13, respectively. The analysis of variance for the four MLN score-sets showed that the germplasms were only significantly different at MLN 3 and MLN 4 (Table 2). Highly significant differences ( $p < 0.001$ ) were also obtained for the AUDPC scores of the germplasms. Germplasms mean of Turkey's multiple comparison range tests at significant levels of 5% denoted by letters A to M showed the lowest and highest MLN scores of 4.7 and 7.5, being GV 662A and ZM 4384, respectively (Table 3). Typical leaf MLN leaf symptoms of infected plants observed in the evaluations are as shown

in Figure 1A to D.

### Area under disease progress curve (AUDPC) for the MLN

The mean AUDPC values of the germplasms showed progressive increase to MLN infection with time (Figure 2). The final MLN infection and the AUDPC values were indicative of the variable reaction of the test germplasm to MLN.

## DISCUSSION

This study evaluated 473 diverse maize germplasms in order to explore their genetic variation as sources of resistance, generate information on their response to MLN and as a rapid response to the serious threat to the food security, income and livelihoods of many smallholder farmers and their families in Zambia. The validity of the study, undertaken under artificial inoculation at Naivasha, is shown by the efficiency of MLN phenotyping under artificial inoculation supported by the heritability values across various trials undertaken at Naivasha, ranging from 0.71 to 0.95 (Boddupalli et al., 2020).

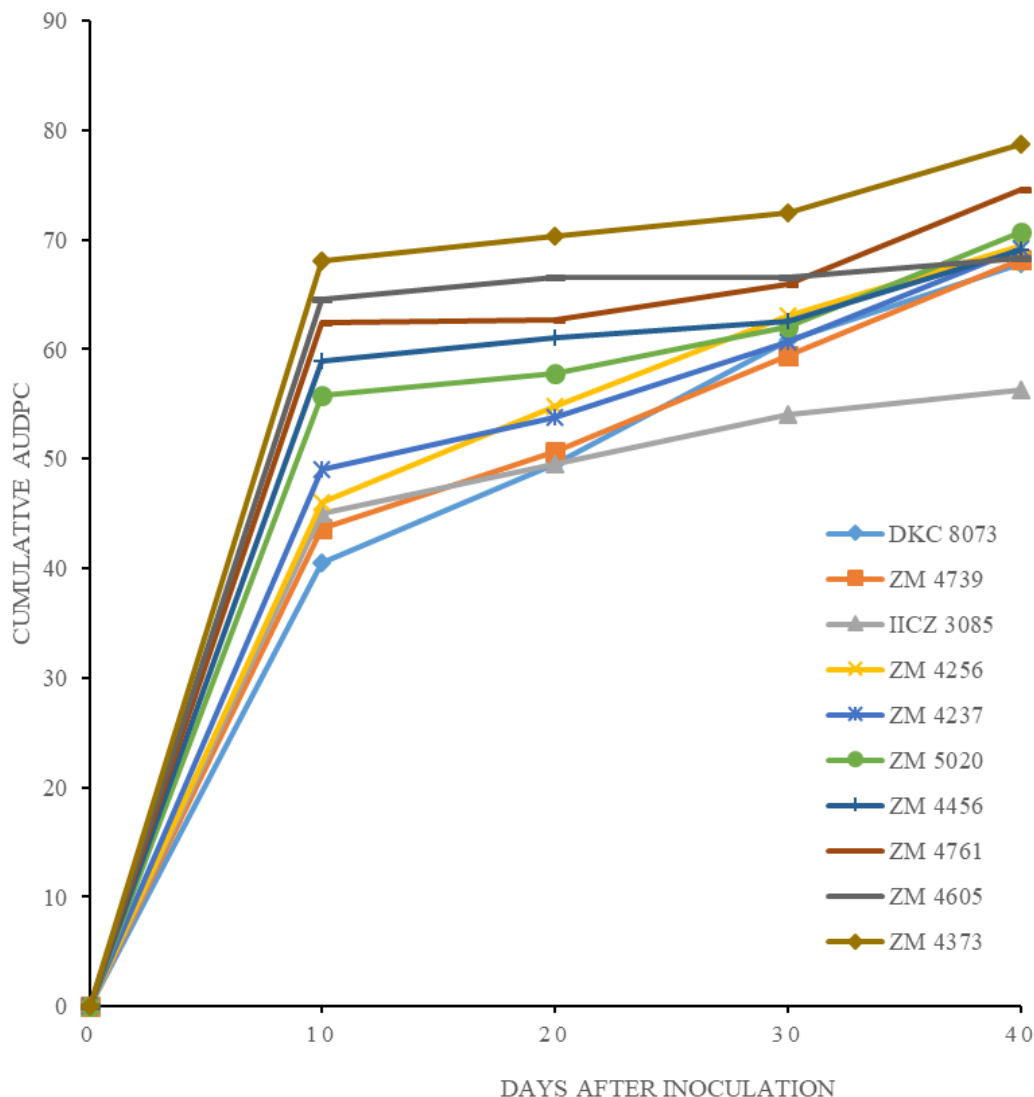
The levels of MLN resistance in the maize germplasms evaluated in this study were very low, with almost all the maize hybrids widely grown in Zambia and accessions held in the national genebank, found to be highly susceptible. In studies consisting of nearly 200,000 germplasms and development of MLN-tolerant/resistant hybrids undertaken by CIMMYT at the MLN screening facility in Naivasha under artificial inoculation, high levels of susceptibility to MLN were shown (Boddupalli et al., 2020; Prasanna, 2015; Semagn et al., 2015). A list of CIMMYT maize inbred lines and pre-commercial hybrids with potential resistance to MLN disease can be obtained

**Table 3.** Germplasms mean (Top 15 and Bottom 15) of Turkey's multiple comparison range tests at significant levels of 5% denoted by letters A to M. Means with the same letter are not significantly different at 5% level.

Germplasm (Top 15)	MLN score	Germplasm (Bottom 15)	MLN score
GV 662A	4.7 <sup>a</sup>	ZM 4307	7.0 <sup>cdefghijklm</sup>
11CZ3085	5.3 <sup>ab</sup>	ZM 6981	7.0 <sup>cdefghijklm</sup>
PAN 4M-19 (LOT #: N/177304/EAD) - F3	5.5 <sup>abc</sup>	ZM 5643	7.0 <sup>cdefghijklm</sup>
ZM 621	5.6 <sup>abcd</sup>	ZM 7354	7.0 <sup>cdefghijklm</sup>
ZMS 722	5.6 <sup>abcd</sup>	NPGRC 4356	7.0 <sup>cdefghijklm</sup>
DKC 9053	5.7 <sup>abcde</sup>	ZM 7348	7.0 <sup>cdefghijklm</sup>
SC 719	5.7 <sup>abcde</sup>	ZM 5189	7.0 <sup>cdefghijklm</sup>
11CZ3087	5.7 <sup>abcde</sup>	ZM 4631	7.1 <sup>cdefghijklm</sup>
NPGRC 4309	5.8 <sup>abcdef</sup>	ZM 8225	7.1 <sup>cdefghijklm</sup>
MRI 514	5.9 <sup>abcdefg</sup>	ZM 4424	7.1 <sup>cdefghijklm</sup>
MRI 744	5.9 <sup>abcdefg</sup>	ZM 4455	7.2 <sup>defghijklm</sup>
DKC 8031	5.9 <sup>abcdefg</sup>	ZM 4348	7.3 <sup>efghijklm</sup>
DKC 8033	5.9 <sup>abcdefg</sup>	ZM 4373	7.4 <sup>fgiklm</sup>
DKC 8073	5.9 <sup>abcdefg</sup>	ZM 4628	7.5 <sup>giklm</sup>
ZM 8226	5.9 <sup>abcdefg</sup>	ZM 4384	7.5 <sup>ikm</sup>



**Figure 1.** Variable MLND reaction types of maize germplasm at mid-whorl growth stage observed during evaluation; fine chlorotic streaks and mottling throughout plant (A); excessive chlorotic mottling and leaf necrosis (B); near complete plant necrosis (C) and field level infection (D).



**Figure 2.** The cumulative AUDPC of MLN on selected top and bottom five germplasm inoculated at 10 day intervals.

at <http://www.cimmyt.org>. Our study had only two maize germplasms, GV 662A and IICZ 3085 showing a moderate level of resistance, with disease scores of 4.7 and 5.3, respectively. According to Boddupalli et al. (2020), for germplasm classification, mean MLN severity scores between 7 and 9 are considered “susceptible”, scores of 5 or 6 as “tolerant”, and scores between 1 and 4 as “resistant”. The current objective of the MLN conversion pipeline is to deliver improved versions of the elite African lines having 0.6 to 1.0 point lower MLN severity score (Semagn et al., 2015). Several attempts have been made to introgress MLN resistance into adapted germplasm, using conventional backcrossing and marker-assisted backcrossing (MABC) leading to a number of first- and second-generation CIMMYT-derived MLN-tolerant hybrids being released on the market in

East Africa (Boddupalli et al., 2020).

In the recent regional MLN surveillance using standardized protocols, current survey data indicates the continued absence of MCMV/MLN in the southern highlands of Tanzania, Malawi, Zambia and Zimbabwe (Boddupalli et al., 2020; <https://mln.cimmyt.org/>). This study provides the first documented experimental evidence undertaken to identify sources of tolerance/resistance to MLN in elite maize germplasms in Zambia and provides information on their status. The development of virus-resistant crops has been shown as an economically viable and environmentally sustainable approach for disease control which requires identification and evaluation of resistant plants and incorporation of favorable alleles into agronomically desirable genetic backgrounds (Semagn et al., 2015).

The on-going strategy for ZARI and other partners will be to continue re-confirming the potential resistance of pre-commercial hybrids and inbred lines that may have the least susceptibility to MLN and work urgently to develop resistant varieties. There is need to look for donor lines that are very tolerant to MLN as well as resistant varieties from CIMMYT and evaluate them for adaptation and release as a short-term measure while long term breeding program incorporating introgressions be put in place. Both the public and private sectors should intensify breeding for tolerance to MLN.

The study has shown that the commonly cultivated varieties in Zambia are susceptible to MLN with exception of GV 662A and IICZ 3085 which were moderately resistant. There is need to enhance the development of MLN tolerant maize cultivars by the national maize breeding programme and seed companies to avert the possible calamity posed by the threat of the effects of MLN. Furthermore, there is need for more intensive awareness creation among the stakeholders in maize value-chain, systematic monitoring and surveillance of MLN, practicing integrated disease management approaches such as avoiding maize monoculture and continuous cultivations of maize throughout the year and practicing maize crop rotation with compatible crops especially legumes that do not serve as hosts for MCMV/MLN.

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## CONFLICT OF INTERESTS

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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