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Response of Assorted Maize Germplasm to the Maize Lethal Necrosis Disease in Kenya

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Abstract

Maize (*Zea mays* L.) is the most widely grown staple food crop in Sub Saharan Africa (SSA) and occupies more than 33 million hectares each year. The recent outbreak and rapid spread of the Maize Lethal Necrosis (MLN) disease has emerged as a great challenge to maize production, threatening food security for the majority of households in the Eastern Africa region with yield loss estimated to be 50-90%. The disease is a result of synergistic interaction between two viruses, *Sugarcane mosaic virus* (SCMV) and *Maize chlorotic mottle virus* (MCMV). The objective of this study was to identify maize genotypes with resistance to MLN. In season one, 73 maize genotypes comprising 25 inbred lines from research institutes, 30 lines from the International Maize and Wheat Improvement Centre (CIMMYT) and 18 farmer varieties were screened for resistance to MLN. In season 2, only 48 genotypes were screened after some of the inbred lines showed complete susceptibility to MLN. These genotypes were grown in three replications in a completely randomized design in polythene bags in the greenhouse at the University of Nairobi. The plants were artificially inoculated using a mixture of SCMV and MCMV. .Weekly MLN disease severity scores using a scale of 1 to 5 (1 = highly resistant and 5 = highly susceptible) and % MLN incidence were recorded and eventually converted into Area under Disease Progress Curve (AUDPC) to give an indication of the disease intensity over time. The plants were allowed to grow to flowering stage to observe the effect of the MLN on the maize productivity. Analysis of Variance revealed wide genetic variation among the genotypes ranging from resistant to highly susceptible. In season 1, three farmer varieties namely MLR2, MLR11 and MLR13 showed resistance to MLN with a mean severity score of 2. In season 2, MLN12, MLN17, MLN18, MLN19, and MLR4 showed low MLN severity ranging from 2-3. The genotypes MLR6, MLR9, MLR16 and MLR18 showed MLN severity of 3 and early maturity traits. This study also validated the presence of MLN resistance among some CIMMYT lines depicted to show resistance in previous studies. These resistant genotypes could serve as donors in the introgression of the resistance into the adapted Kenyan maize backgrounds. This will go a long way in ensuring sustainable maize productivity while improving the livelihoods of the small-scale farmers who form the bulk of the major maize producers in Kenya.

Keywords: maize lethal necrosis disease, MLN severity, maize genotypes

1. Introduction

In Kenya, food security is synonymous with maize availability since it is a key staple food to over 90% of her population with about 42 dietary energy intakes (Keya and Rubaihayo, 2013). The recent outbreak and rapid spread of the Maize Lethal Necrosis (MLN) disease has emerged as a great challenge to maize production, threatening food security for the majority of households in the region. Maize lethal necrosis is a serious disease of maize, and since its first appearance in Kenya in 2011 (Wangai *et al*., 2012), the disease has spread to many countries in the East Africa region where maize is grown including Tanzania, Uganda, South Sudan and Rwanda (IITA, 2014). The disease has been identified as the most devastating foliar disease responsible for highest yield loss in maize because it causes the yield loss of up to 100% because of its ability to kill infected plant and cells (Mbega *et al*., 2016).

MLN is caused by a mixed or synergistic infection between *Maize chlorotic mottle virus* (MCMV, genus Machlomovirus) and potyviruses infecting maize which includes either *Sugarcane mosaic virus* (SCMV) or

Maize dwarf mosaic virus (MDMV) and *Wheat streak mosaic virus* (WSMV). The synergism between viruses refers to situations where co-infection with two viruses leads to more virulence as opposed to singular infections. It is also a state where multiplication of at least one of the viruses is enhanced by the other (Shi *et al*., 1997; Karyeija *et al*., 2000). The MCMV belongs to the genus Machlomovirus of the family Tombusviridae. It was first identified in maize in Peru in 1974 and USA in 1978 and 1980 (Wu *et al*., 2013). It is most common in the graminae family. The SCMV causes mosaic diseases and consists of four distinct Potyviruses including strains of *Johnsongrass mosaic virus* (JGMV), MDMV, *Sorghum mosaic virus* (SrMV) and SCMV (Yang and Mirkov, 1997). The MCMV causes mild mosaic, severe stunting, leaf necrosis, premature plant death, shortened male inflorescences with few spikes, and shortened, malformed and partially filled ears (Wu *et al*., 2013). *Sugarcane mosaic virus* and MDMV are two important pathogens of maize and related crops, causing yield losses, chlorosis and stunting (Xu *et al*., 2000). The infected maize plants are frequently barren, the ears formed are small, deformed and set little or no seeds, drastically reducing the yield. This greatly affects the physiological processes like photosynthesis and chlorophyll formation (Wangai *et al*., 2012), causing failure of tasseling or sterility in male plants. These could also lead to deformed or no ears or even rotting of the cobs (Adams *et al*., 2013).The MLN disease also predisposes the plants to secondary fungal infections (FSNWG, 2012; Wu *et al*., 2013). The type of virus, plant phenology, time of infection, host plant growth conditions and genotype all influence the extent of disease spread and distribution (Melchinger *et al*., 1998). The disease is also aggravated by drought conditions and poor soil fertility (http://www.fao.org/2012). Other predisposing factors include the growing of host crops like sugarcane and millet that contributes towards increased inoculum loads. Insect vectors also aid in the transmission of viruses e.g. maize thrips and beetles have been associated with the transmission of MCMV while aphids transmit SCMV (Cabanas *et al*., 2013; Wu *et al*., 2013). Other forms of transmission include either mechanical or through the seed (Miano and Kabaki, 2013; Wu *et al*., 2013).

The MLN challenge is compounded by the fact that the viruses inhabit leaves, pollen, female and male inflorescences, ear husks, cotyledons and seeds (Nelson *et al*., 2011) further complicating MLN disease management. A gene GRMZM2G018943 was reported to function as the translation initiation factor eIF-2B and this was associated with the mutation of plant proteins which aims at countering the viral attack (Ingvardsen *et al*., 2010). However, this seems not to be the case especially among the currently grown maize varieties in Kenya which have shown high susceptibility to MLN. The nature of entry and replication of the SCMV virus into the plants also aggravates the MLN menace (Ingvardsen *et al*., 2010; Gowda et al., 2015). Knowledge of the virus dynamics could offer a more reliable approach towards management of the MLN epidemics (Redinbaugh *et al*., 2000). The management of MLN disease could be achieved through integrating cultural methods, chemical method such as seed dressing and foliar spray with host resistance breeding. In Kenya, poor agricultural practices like leaving infected maize crop residues in the field and maize monoculture do not aid in breaking the disease transmission cycle. This implies that insect vectors transmit the viruses through crops and seasons. Lack of proper weed management practices has also aided in offering alternative hosts to these vectors since these weeds are susceptible to the MCMV. The use of chemicals is uneconomical and environmentally unfriendly especially among the resource constrained small-scale maize farmers. These chemicals are rendered ineffective due to the non-persistent transmission of the viruses (Melchinger *et al*., 1998).

Host breeding for resistance is the most ecologically safe and economical approach towards combating the MLN disease menace. Previous breeding efforts by the International Maize and Wheat Improvement Centre (CIMMYT) and Kenya Agricultural and Livestock Research Organization (KALRO) have reported very high susceptibility of around 90% to MLN disease among the pre-commercial and commercial maize germplasm (Gowda *et al*., 2015). This implies that reduction in maize production due to the impact of MLN disease will and has already adversely affected the Kenyan livelihoods and the overall market prices of maize (Wangai *et al*., 2012). The areas affected constitute major maize production acreage and given the recorded loss of up to 100%, it has become an important food security issue in Kenya. The impact of the disease has been felt in the whole maize value chain.

Efforts put in breeding to improve maize productivity are aimed at developing many varieties which are resistant to both biotic and abiotic stresses. CIMMYT has undertaken discovery studies to identify genomic regions associated with MLN disease resistance where two mapping panels and six bi-parental populations have revealed three major QTLs on chromosomes and a few minor QTLs across other chromosomes (Olsen *et al*., 2016). These QTLs are aimed for introgression into adapted maize genotypes. The identification of new sources of resistance could contribute valuable alleles which may supplement the ones in use. The objective of this study was to determine the response of assorted maize genotypes to MLN infection and identify any heterotic parents which could be utilized in maize breeding programs to improve maize productivity in the region.

2. Materials and Methods

2.1 Germplasms

The maize genotypes were assembled from diverse sources namely CIMMYT, KALRO and farmers' varieties (landraces) (Table 1).

2.2 Experiment Site Description

The experiments were done in the greenhouse located at the University of Nairobi Field Station (Kabete) for two seasons. Kabete is situated at Latitude of 1^o 15′S and Longitude 36^o 44′E, and at an altitude 1930 m above sea level. Soils are usually humic nitisol, well drained, deep (>180cm), dark red to darkish brown. Kabete has a bimodal distribution of rainfall, with long rains from early March to late May and the short rains from October to December. The mean annual temperature is 18^o C and the mean annual rainfall is 1006 mm (Onyango *et al*., 2012).

2.2.1 Experiment Design and Layout

The experiment was set up in a completely randomized design in three replications. The genotypes were grown in polythene bags measuring 20 cm in diameter in the greenhouse. Previous studies have validated the use of greenhouse due to the fact that the plants are tender leading to efficacy in inoculations and clear genotype diagnosis (Melchinger *et al*., 1998). All the agronomic practices were observed namely; hand weeding, irrigation, fertilizer application whereby DAP was used during planting at the rate of 10 g/hill while urea was used as a top dressing fertilizer. After 14 days post germination, the genotypes were artificially inoculated using a combination of both viruses namely MCMV and SCMV through hand rubbing.

2.2.2 Isolation of the Pathogen

The MLN causal viruses namely MCMV SCMV were isolated from diseased tissue of maize leaves showing clear virus symptoms at KALRO where the two viruses are maintained at the Biosafety Greenhouse (BGH).

2.2.3 Preparation of Inocula and Inoculation

The leaves were cut into small pieces and stored in the freezer at a temperature of -20^0 C. Phosphate buffer 0.1 M was made by mixing potassium phosphate Dibasic (Anhydrous) and Potassium dihydrogen orthophosphate (Potassium phosphate monobasic) to a pH of 7.0 using the following ratios; KH_2PO4 = 10.8g, K_2HPO4 = 4.8g and Na_2SO3 = 1.26 and Carborandum (SiCO3) = 1g/l. Then 2g of leaves with MCMV and 10g of leaves with SCMV at a ratio of 1:5 were weighed and ground using sterile mortar and pestle to obtain homogenate solution or extract. The extract was added to the buffer to make 120 ml. The combination of MCMV and SCMV inoculums was then rubbed onto two week old young leaves of the maize seedlings in the greenhouse. The Carborandum powder ($SiCO_3$) which is an abrasive agent was used to cause microscopic injury of the leaves for easy penetration of the virus into the plant cells (Orawu *et al*., 2013). A second inoculation was done at the interval of one week from the first inoculation to ensure effective viral dissemination and spread among the genotypes and that there were no diseases escapes.

Table 1. List of the genotypes used for screening and their sources

UoN Designation	Genotype	Origin	Season 1	Season 2
UoN-2015-51	KAT25-1	KALRO	1	-
UoN-2015-52	KAT25-2	KALRO	1	-
UoN-2015-53	KAT25-3	KALRO	1	-
UoN-2015-54	KAT25-4	KALRO	1	-
UoN-2015-55	KAT25-5	KALRO	1	-
UoN-2015-56	KAT25-6	KALRO	1	-
UoN-2015-57	KAT25-7	KALRO	1	-
UoN-2015-58	KAT25-8	KALRO	1	-
UoN-2015-59	KAT25-9	KALRO	1	-
UoN-2015-60	KAT25-10	KALRO	1	-
UoN-2015-61	KAT25-11	KALRO	1	-
UoN-2015-62	KAT25-12	KALRO	1	-
UoN-2015-63	KAT25-13	KALRO	1	-
UoN-2015-64	KAT25-14	KALRO	1	-
UoN-2015-65	KAT25-15	KALRO	1	-
UoN-2015-66	KAT25-16	KALRO	1	-
UoN-2015-67	KAT25-17	KALRO	1	-
UoN-2015-68	KAT25-18	KALRO	1	-
UoN-2015-69	KAT25-19	KALRO	1	-
UoN-2015-70	KAT25-20	KALRO	1	-

UoN Designation	Genotype	Origin	Season 1	Season 2
UoN-2015-71	KAT25-21	KALRO	1	-
UoN-2015-72	KAT25-22	KALRO	1	-
UoN-2015-73	KAT25-23	KALRO	1	-
UoN-2015-74	KAT25-24	KALRO	1	-
UoN-2015-75	KAT25-25	KALRO	1	-
UoN-2015-76	MLN1	CIMMYT	1	2
UoN-2015-77	MLN2	CIMMYT	1	2
UoN-2015-78	MLN3	CIMMYT	1	2
UoN-2015-79	MLN4	CIMMYT	1	2
UoN-2015-80	MLN5	CIMMYT	1	2
UoN-2015-81	MLN6	CIMMYT	1	2
UoN-2015-82	MLN7	CIMMYT	1	2
UoN-2015-83	MLN8	CIMMYT	1	2
UoN-2015-84	MLN9	CIMMYT	1	2
UoN-2015-85	MLN10	CIMMYT	1	2
UoN-2015-86	MLN11	CIMMYT	1	2
UoN-2015-87	MLN12	CIMMYT	1	2
UoN-2015-88	MLN13	CIMMYT	1	2
UoN-2015-89	MLN14	CIMMYT	1	2
UoN-2015-90	MLN15	CIMMYT	1	2
UoN-2015-91	MLN16	CIMMYT	1	2
UoN-2015-92	MLN17	CIMMYT	1	2
UoN-2015-93	MLN18	CIMMYT	1	2
UoN-2015-94	MLN19	CIMMYT	1	2
UoN-2015-95	MLN20	CIMMYT	1	2
UoN-2015-96	MLN21	CIMMYT	1	2
UoN-2015-97	MLN22	CIMMYT	1	2
UoN-2015-98	MLN23	CIMMYT	1	2
UoN-2015-99	MLN24	CIMMYT	1	2
UoN-2015-100	MLN25	CIMMYT	1	2
UoN-2015-101	MLN26	CIMMYT	1	2
UoN-2015-102	MLN27	CIMMYT	1	2
UoN-2015-103	MLN28	CIMMYT	1	2
UoN-2015-104	MLN29	CIMMYT	1	2
UoN-2015-105	MLN30	CIMMYT	1	2
UoN-2015-106	MLR1	Farmer varieties	1	2
UoN-2015-107	MLR2	Farmer varieties	1	2
UoN-2015-108	MLR3	Farmer varieties	1	2
UoN-2015-109	MLR4	Farmer varieties	1	2
UoN-2015-110	MLR5	Farmer varieties	1	2
UoN-2015-111	MLR6	Farmer varieties	1	2
UoN-2015-112	MLR7	Farmer varieties	1	2
UoN-2015-113	MLR8	Farmer varieties	1	2
UoN-2015-114	MLR9	Farmer varieties	1	2
UoN-2015-115	MLR10	Farmer varieties	1	2
UoN-2015-116	MLR11	Farmer varieties	1	2
UoN-2015-117	MLR12	Farmer varieties	1	2
UoN-2015-118	MLR13	Farmer varieties	1	2
UoN-2015-119	MLR14	Farmer varieties	1	2
UoN-2015-120	MLR15	Farmer varieties	1	2
UoN-2015-121	MLR16	Farmer varieties	1	2
UoN-2015-122	MLR17	Farmer varieties	1	2

UoN= University of Nairobi; KALRO=Kenya Agricultural and Livestock Research Organization; CIMMYT=International Maize and Wheat Improvement Centre; - = not evaluated in season two

2.3 Data Collection

The MLN symptoms were assessed for disease severity based on the CIMMYT scale (Table 2) and percent MLN incidence. Disease severity scoring began one week after the repeat inoculation and this was done weekly for eight weeks. The delayed scoring for the presence of MLN was to detect late developing infections (Zambrano *et al*., 2013). The plants were allowed to grow to physiological maturity to enable one to get an indication of the effect of MLN on maturity and yielding potential. The percentage MLN incidence was measured as the percentage of the number of leaves with MLN infection.

Table 1. CIMMYT scale used in assessment of the MLN disease

Score	Symptoms
1	No MLN symptoms
2	Fine chlorotic streaks
3	Chlorotic mottling
4	Excessive chlorotic mottling and some necrosis
5	Dead heart symptoms/ complete plant death

Source (CIMMYT).

2.4 Data Analysis

The disease severity scores were converted into the Area Under Disease Progress Curve (AUDPC) values based on Wilcoxson *et al*. (1975). AUDPC is simply the intensity of disease integrated between two times. It is a crucial quantitative summary of the disease intensity over time for comparison across years, locations as well as management tactics. The AUDPC expresses the dynamics of an epidemic as a single value and different epidemics can be compared by normalizing the different AUDPC value of each epidemic by calculating the relative area under disease progress curve (AUDPC) (Equation (1) (Wilcoxson *et al*., 1975).

$$\text{AUDPC} = \sum_{i=1}^{n-1}\left(\frac{yi+yi+1}{2}\right)(ti+1-ti) \qquad \text{Equation 1} \qquad (1)$$

Whereby,

n= total number of observations, y_i= injury intensity (usually incidence in crop health data) at the *i*th observation, *t* = time at the *i*th observation.

2.4.1 Analysis of Variance

The AUDPC values, the weekly MLN severity scores, % MLN incidence and other agronomic traits were subjected to the analysis of variance (ANOVA). The genotype means were separated using Fisher's protected least significant differences (LSD) test at 5% significance level.

2.4.2 Correlation among the Different Traits

A Pearson's Correlation Coefficient to establish the phenotypic relationship among the different disease parameters assessed was done following the formula by Pearson (1896) shown on Equation (2).

$$r = \frac{\sum(X-\bar{X})(Y-\bar{Y})}{\sqrt{\quad}\ \sqrt{\quad}} \qquad (2)$$

Where,

$\bar{X}$= mean of X variable; $\bar{Y}$= mean of Y variable

3. Results

3.1 Analysis of Variance

The findings revealed that there were significant differences among the genotypes for the different MLN disease parameters at $P<0.05$. The replications were not significantly different implying that these findings were repeatable and reproducible through seasons (Table 3 and Table 4).

3.2 Mean Performance of the Assorted Maize Germplasm across Two Seasons

The mean performance of the maize genotypes under MLN artificial infection varied significantly across the two seasons (Table 5). During season 1, 73 genotypes were screened for resistance to MLN whereas in season 2, 50 genotypes were assessed for MLN resistance. The response of the genotypes to the MLN was assessed based on the parameters AUDPC, final disease severity (FS) and MLN disease incidence. To obtain absolute values, there was the use of the relative Final MLN severity (rFS) scores and relative Area Under Disease Progress Curve (rAUDPC).

The genotypes assembled from CIMMYT showed good resistance to the MLN especially during season 2 despite the heavy MLN disease pressure. The genotypes were also divergent with respect to either earliness or lateness in maturity with most of the KALRO and farmer varieties showing the earliness trait. Most of the CIMMYT derived genotypes showed lateness trait.

In season 1, the MLN severity scores ranged from 1 to 5 among the genotypes. The AUDPC had a range of 76 to 148 while the % MLN incidence had a range of 5 to 100%. During season 2, the MLN severity scores ranged from 2 to 5 among the genotypes. The AUDPC had a range of 109 to 246 while the % MLN incidence had a

range of 26 to 100%.

In season one, twenty of the genotypes which showed high MLN resistance also had earliness trait namely KAT25-10, KAT25-16, MLN1, MLN15, MLN16, MLR-10, MLR-13 and MLR-17 with MLN scores ranging from 1 to 2 (Table 4). In season 2, MLN17, MLN19, MLR4, MLN18 and MLN12 combined good MLN disease parameters with MLN severity ranging from 2-3 and also statistically low AUDPC, rAUDPC and % MLN incidence values. These genotypes also exhibited lateness in flowering with statistically many days to 50% tasseling and silking. The genotypes MLR6, MLR9, MLR16 and MLR18 showed MLN severity of 3 and early maturity with statistically few number of days to both 50% tasseling and silking.

3.3 Correlations among Traits in Season across the Two Seasons

The MLN disease parameters namely AUDPC, FS and % DI showed positive and highly significant correlation coefficient values validating their use in estimating the most susceptible and most MLN resistant genotype (Table 7). The weekly MLN progressions also showed positive and highly significant correlation coefficients implying that the period of MLN evaluation was sufficient. However, no distinct relationship was established between the maturity indicators namely days to 50% pollen shed and silking and the MLN assessment parameters.

Table 3. Mean Squares for the MLN disease parameters during season 1

Source of variation	df	% incidence	MLN severity	AUDPC	rAUDPC	rFS
Replications	2	4444.90	3.90	2316.70	882.70	3.90
Genotypes	71	1879.4*	1.5*	993.3*	378.5*	1.5*
Residual	124	846.90	0.50	380.60	145.00	0.50
Total	197					

Table 4. Mean squares for the MLN disease parameters during season 2

Source of variation	**df**	**% MLN incidence**	**MLN severity**	**AUDPC**	**rAUDPC**	**rFS**
Replications	2	24240.80	8.27	46273.00	5210.70	17.33
Entry	46	10562.7*	5.43*	21743*	2448.4*	6.3*
Residual	68	526.90	0.49	1165.00	131.20	0.58
Total	116					

*=Significant difference at 5%; MLN Severity: was assessed based on CIMMYT scale (1-5) where 1 = No MLN symptoms and 5 = Dead heart symptoms/ complete plant death; AUDPC: area under the disease progress curve calculated from the weekly MLN severity scores based on Wilcoxson *et al*. (1975) equation; rAUDPC: relative Area Under Disease Progress Curve was calculated to obtain absolute values where the most MLN susceptible genotype was used for computation; % MLN incidence: percentage of the number of leaves with MLN infection

Table 5. Weekly MLN disease progression in season 1 and MLN disease parameters

Entry	**Genotypes**	**Weekly MLN severity scores (1 – 5)**								**AUDPC**	**rAUDPC**	**% MLN incidence**	**rFS**	**DTS**	**DTP**
		week1	**week2**	**week3**	**week4**	**week5**	**week6**	**week7**	**week8**						
1	KAT25-1	1.67	2.00	2.33	3.00	3.33	3.67	3.67	4.67	146.00	90.12	100.00	4.67	58.08	64.53
2	KAT25-2	1.33	2.00	2.67	3.00	3.67	3.67	3.67	4.33	148.20	91.46	100.00	4.33	62.59	70.53
3	KAT25-3	0.67	1.67	2.67	2.33	3.00	3.00	3.00	3.00	120.30	74.28	100.00	3.00	59.08	60.33
4	KAT25-4	0.67	2.00	3.00	3.00	3.33	3.00	3.00	3.33	132.80	82.00	86.70	3.33	61.81	67.00
5	KAT25-5	1.00	1.67	2.33	2.33	2.67	2.33	2.33	3.33	108.80	67.18	79.20	3.33	71.77	70.53
6	KAT25-6	1.67	2.00	2.33	2.67	2.33	2.67	2.67	3.00	116.80	72.12	76.80	3.00	62.59	71.19
7	KAT25-7	1.67	2.00	2.00	2.00	2.33	2.00	2.33	2.67	101.80	62.86	66.10	2.67	63.67	55.67
8	KAT25-8	0.94	1.94	1.44	1.44	1.44	1.94	1.44	1.94	75.60	46.68	15.20	1.87	63.31	51.78
9	KAT25-9	0.67	1.67	1.33	1.67	2.33	2.33	3.00	2.67	96.50	59.57	69.40	2.67	66.77	64.00
10	KAT25-10	2.04	2.04	1.04	2.04	2.54	2.54	2.04	2.54	100.60	62.10	67.20	2.57	55.61	51.00
11	KAT25-11	1.33	1.67	2.00	2.67	2.67	2.33	2.00	2.33	104.30	64.40	44.20	2.33	70.83	67.33
12	KAT25-12	0.67	2.00	2.00	2.33	2.00	1.67	1.00	1.00	80.80	49.90	4.80	1.00	62.59	71.00
13	KAT25-13	1.33	1.33	2.00	2.33	2.00	2.00	1.33	1.33	84.70	52.26	6.70	1.33	62.59	71.19
14	KAT25-14	1.02	1.52	1.52	2.02	2.52	2.02	2.02	2.02	90.30	55.73	51.50	2.06	65.67	54.67
15	KAT25-15	1.67	2.33	2.00	2.67	2.67	3.00	3.00	3.00	123.80	76.44	93.60	3.00	59.58	53.03
16	KAT25-16	1.04	2.04	2.04	2.04	2.54	2.54	2.04	2.04	101.80	62.87	41.80	2.07	58.33	60.00
17	KAT25-17	1.33	2.00	2.67	2.67	3.00	3.00	3.00	3.00	127.20	78.50	88.90	3.00	63.31	53.78
18	KAT25-18	1.00	2.00	1.67	2.67	2.67	2.67	2.67	3.00	112.50	69.44	96.70	3.00	58.00	76.00
19	KAT25-19	1.67	1.33	1.67	2.00	2.33	2.00	1.67	1.67	87.20	53.81	17.40	1.67	63.11	51.69
20	KAT25-20	1.02	2.02	2.52	2.52	3.02	4.02	3.91	4.90	139.50	86.12	93.80	4.85	70.61	60.69
21	KAT25-21	1.67	2.00	2.00	2.67	2.67	2.67	2.67	2.67	115.80	71.50	70.40	2.67	65.81	71.00

Entry	Genotypes	Weekly MLN severity scores (1 – 5)								AUDPC	rAUDPC	% MLN incidence	rFS	DTS	DTP
		week1	week2	week3	week4	week5	week6	week7	week8						
22	KAT25-22	1.67	2.00	3.00	3.00	3.33	3.33	3.33	4.33	144.50	89.20	100.00	4.33	62.59	70.60
23	KAT25-23	1.67	2.00	2.00	2.33	2.67	2.33	2.00	2.33	105.30	65.02	68.30	2.33	62.59	70.43
24	KAT25-24	1.00	2.00	2.00	2.00	2.00	2.33	2.33	2.67	99.50	61.42	54.20	2.67	62.59	71.92
25	KAT25-25	0.67	2.00	2.33	3.00	2.67	2.67	2.67	2.67	116.80	72.12	60.70	2.67	65.81	58.00
26	MLN1	1.02	2.02	1.52	1.52	2.02	2.52	2.02	2.02	90.00	55.58	18.90	2.06	54.85	60.78
27	MLN2	1.67	1.33	1.67	2.00	2.67	2.67	2.67	2.33	103.50	63.89	61.10	2.33	59.84	77.90
28	MLN3	1.33	1.67	2.00	2.00	2.00	2.00	2.00	2.33	90.30	55.76	56.80	2.33	71.77	67.28
29	MLN4	0.67	2.00	2.00	2.00	2.67	2.67	2.33	2.33	104.20	64.30	66.20	2.33	65.38	63.69
30	MLN5	1.00	1.67	1.00	1.67	2.33	2.00	2.00	3.00	87.30	53.91	69.10	3.00	62.59	67.92
31	MLN6	1.44	1.94	2.44	1.94	2.94	2.44	2.44	2.44	110.10	67.98	53.30	2.37	67.08	56.53
32	MLN7	1.67	1.67	1.00	1.33	2.33	2.33	2.67	3.00	94.30	58.23	100.00	3.00	54.85	74.91
33	MLN8	1.54	2.04	2.54	2.54	3.04	3.04	3.04	3.04	127.80	78.92	89.30	3.07	61.00	66.00
34	MLN9	1.33	1.67	1.00	1.33	2.00	2.00	1.67	1.33	75.70	46.71	9.10	1.33	70.83	64.78
35	MLN10	1.33	2.33	2.00	2.33	2.67	2.67	2.67	2.67	114.50	70.68	63.30	2.67	70.83	65.78
36	MLN11	0.67	1.67	2.00	2.33	3.00	3.33	3.00	3.00	118.30	73.05	77.80	3.00	59.00	71.33
37	MLN12	1.33	1.67	1.33	2.00	2.00	2.00	2.00	2.00	87.20	53.81	13.70	2.00	62.59	64.85
38	MLN13	1.33	2.00	1.67	2.00	2.33	2.00	2.00	2.33	95.00	58.64	55.50	2.33	65.84	70.43
39	MLN14	1.54	2.04	2.54	3.04	3.04	2.54	3.54	4.04	134.80	83.24	95.30	4.07	57.61	66.00
40	MLN15	1.23	1.82	1.92	2.24	2.58	2.56	2.51	2.66	107.10	66.13	63.20	2.66	59.33	50.00
41	MLN16	1.00	1.00	1.00	1.67	1.67	2.00	2.00	2.33	76.00	46.91	53.40	2.33	56.61	63.69
42	MLN17	1.33	1.33	1.67	1.67	2.00	2.33	2.33	2.33	90.70	55.97	52.40	2.33	54.85	72.78
43	MLN18	0.67	1.33	1.33	2.00	2.33	2.33	2.33	2.33	90.80	56.07	50.40	2.33	55.79	74.28
44	MLN19	1.33	1.33	1.67	1.33	2.33	2.67	2.33	2.00	91.80	56.69	51.10	2.00	62.59	69.19
45	MLN20	1.00	2.33	1.33	2.00	2.00	2.33	2.00	2.00	92.70	57.20	31.20	2.00	59.39	70.33
46	MLN21	1.00	2.00	2.00	1.67	3.33	2.67	3.00	3.00	114.70	70.78	62.10	3.00	64.58	66.33
47	MLN22	1.04	2.04	1.54	2.54	2.54	2.54	2.04	2.04	102.10	63.02	48.00	2.07	60.00	67.00
48	MLN23	1.67	2.00	2.00	2.67	3.00	3.00	3.00	3.00	124.00	76.54	89.80	3.00	55.79	73.60
49	MLN24	1.33	2.00	2.33	2.00	2.33	2.67	2.67	2.67	109.80	67.80	62.80	2.67	70.37	68.53
50	MLN25	1.00	2.00	2.00	2.67	3.00	3.00	3.00	3.00	121.70	75.10	70.10	3.00	70.61	67.67
51	MLN26	1.33	1.67	1.67	2.33	2.33	2.33	2.00	2.00	96.30	59.47	38.50	2.00	56.78	69.78
52	MLN27	1.33	1.33	1.00	1.33	2.00	2.00	2.33	2.67	82.80	51.13	67.90	2.67	62.59	70.43
53	MLN28	1.00	1.67	1.33	2.00	2.33	2.33	2.67	3.00	98.80	61.01	83.60	3.00	55.31	74.00
54	MLN29	2.00	2.00	1.33	2.00	2.67	2.67	2.67	2.33	106.80	65.95	41.20	2.33	62.84	67.92
55	MLN30	1.67	1.67	1.33	2.00	2.33	2.00	1.67	1.67	87.20	53.81	23.90	1.67	70.37	63.45
56	MLR-1	1.00	2.00	2.33	2.33	2.67	3.04	2.54	3.04	119.10	73.52	74.90	3.07	63.78	72.28
57	MLR-2	1.00	1.67	1.00	1.33	1.67	2.00	2.00	2.00	76.80	47.43	45.40	2.00	70.61	68.33
58	MLR-3	2.04	2.04	3.04	3.04	3.04	2.54	3.54	4.04	139.80	86.32	93.60	4.07	59.39	63.69
59	MLR-4	2.00	2.00	2.67	3.33	3.33	3.00	3.33	3.00	138.80	85.70	90.60	3.00	70.83	65.69
60	MLR-5	1.94	1.44	2.44	2.94	2.94	2.94	2.94	2.94	124.40	76.77	76.70	2.87	59.00	54.67
61	MLR-6	1.00	1.67	2.33	2.67	3.00	2.67	2.67	2.67	115.80	71.50	73.60	2.67	60.78	61.53
62	MLR-7	0.44	1.94	1.44	1.44	2.44	2.44	2.44	2.94	94.90	58.56	68.90	2.87	64.81	62.67
63	MLR-8	1.33	1.67	2.33	2.67	2.67	2.67	2.67	2.33	113.50	70.06	56.40	2.33	60.00	70.33
64	MLR-9	1.00	2.00	2.00	2.67	2.67	3.00	3.00	3.00	119.30	73.66	85.60	3.00	64.58	56.53
65	MLR-10	0.92	1.92	1.92	1.92	2.91	2.91	2.91	2.91	112.30	69.31	74.00	2.86	60.31	50.28
66	MLR-11	0.67	1.33	1.33	2.00	2.00	2.00	2.00	2.00	82.70	51.03	29.70	2.00	68.11	63.67
67	MLR-12	1.54	1.54	1.54	2.04	2.54	2.54	2.04	2.54	98.80	61.02	58.50	2.57	58.00	68.00
68	MLR-13	0.67	2.00	1.67	2.00	2.33	2.33	2.33	2.33	97.30	60.08	53.90	2.33	59.39	61.53
69	MLR-14	0.67	1.67	2.00	2.67	3.00	3.00	3.33	3.33	121.80	75.21	93.90	3.33	62.59	70.60
70	MLR-15	0.67	1.67	2.33	2.00	2.67	2.67	3.00	2.67	110.00	67.90	75.40	2.67	59.00	62.33
71	MLR-16	1.33	1.67	2.33	3.00	3.00	2.67	2.67	2.67	119.30	73.66	75.00	2.67	62.08	56.03
72	MLR-17	1.33	2.00	2.33	2.67	2.67	2.67	2.67	2.00	116.80	72.12	63.60	2.00	62.59	60.94
73	check	1.33	2.33	2.00	2.33	2.67	2.67	2.33	2.33	111.00	68.52	50.00	2.33	62.33	50.00
Least significant difference										31.5	19.5	47.0	1.2	8.19	14.65
%Coefficient of variation										5.3	5.3	12.4	8.8	1.10	1.30

*=Significant difference at 5%; MLN Severity: was assessed based CIMMYT scale (1-5) where 1 = No MLN symptoms and 5 = Dead heart symptoms/ complete plant death; AUDPC: area under the disease progress curve calculated from the weekly MLN severity scores based on Wilcoxson *et al.* (1975) equation; rAUDPC: relative Area Under Disease Progress Curve calculated as a percentage of the AUDPC; % MLN incidence: percentage of the number of leaves with MLND infection; DTS= Days to 50% silking, DTP= Days to 50% Pollen shed

Table 6. Weekly MLN disease progression in season 2 and other MN disease parameters

Entry	Genotype	Weekly MLN severity scores (1 – 5)							AUDPC	rAUDPC	FS	rFS	%MLN	DTP	DTS
		week1	week2	week3	week4	week5	week6	week7					incidence		
1	MLN1	1.09	1.09	2.09	2.09	2.09	2.09	4	143	48	4	4	43	93.5	89.1
2	MLN2	1.92	1.92	2.92	2.92	2.92	2.92	4	187	63	4	4	97	85.3	78.6
3	MLN3	2.00	2.00	2.00	2.00	2.33	3.00	4	172	58	4	4	98	92.7	87.3
4	MLN4	2.00	2.00	2.00	2.00	2.00	3.00	4	167	56	4	4	81	90	83
5	MLN5	2.00	2.00	2.00	2.00	2.00	3.00	4	176	59	4	4	97	89.5	90
6	MLN6	1.09	1.09	1.09	1.09	1.09	2.59	3	130	44	3	3	63	84	80.6
7	MLN7	1.92	2.92	3.42	3.42	3.42	4.42	4	241	81	4	4	97	85.8	97.3
8	MLN8	1.16	2.16	2.16	2.16	3.16	3.16	3	179	60	3	3	83	85.8	80.9
9	MLN9	1.33	1.33	1.33	1.33	1.67	2.67	3	135	45	3	3	55	88.7	83
10	MLN10	1.59	2.09	2.09	2.09	2.09	3.09	3	162	54	3	3	69	85.4	85.6
11	MLN11	2.33	3.00	3.00	3.00	3.33	3.67	4	217	73	4	4	100	84	84.3
12	MLN12	1.01	1.01	1.01	1.01	2.01	2.01	3	123	41	3	3	26	93.7	94.3
13	MLN13	2.09	2.09	2.09	2.09	3.09	3.59	4	193	65	4	4	100	87.5	85.1
14	MLN14	1.66	1.91	2.04	2.04	2.27	2.94	3	165	56	3	3	74	85.8	80.9
15	MLN15	1.50	1.50	1.50	1.50	2.00	2.50	3	147	49	4	4	47	92.1	87.8
16	MLN16	3.16	3.16	3.16	3.16	3.16	4.16	4	239	80	4	4	74	85.8	91.9
17	MLN17	1.50	1.50	1.50	1.50	1.50	2.50	2	126	42	3	3	27	92.6	89.8
18	MLN18	0.92	1.42	1.42	1.42	1.42	2.42	3	126	42	3	3	34	92.3	89.1
19	MLN19	1.33	1.33	1.67	1.67	1.67	2.33	3	127	43	3	3	38	95	92.7
20	MLN20	1.92	1.92	1.92	1.92	2.42	3.42	3	175	59	3	3	97	88.3	89.1
21	MLN21	2.00	2.33	2.33	2.33	2.67	3.33	4	191	64	4	4	75	86	82
22	MLN22	1.01	1.01	2.01	2.01	2.01	3.01	4	162	54	4	4	80	93.7	90.3
23	MLN23	1.66	1.91	2.04	2.04	2.27	2.94	3	165	56	3	3	74	85.8	80.9
24	MLN24	1.59	2.09	2.09	2.09	2.09	2.59	3	153	51	3	3	66	93.3	83.6
25	MLN25	1.33	1.67	2.00	2.00	2.33	2.67	3	147	49	3	3	61	88.7	82
26	MLN26	1.33	2.33	2.33	2.33	2.67	3.00	3	169	57	3	3	79	86.8	81.7
27	MLN27	1.67	2.00	2.00	2.00	2.00	2.67	3	151	51	3	3	69	90	84
28	MLN28	1.50	1.50	1.50	1.50	2.00	2.00	3	131	44	3	3	56	90.9	87.8
29	MLN29	1.00	1.33	1.33	1.33	1.33	2.00	2	109	36	2	2	51	88.7	85.3
30	MLN30	1.33	2.00	2.00	2.00	2.00	3.00	3	158	53	3	3	94	90.3	84
31	MLR1	1.33	1.33	1.67	1.67	1.67	2.33	3	137	46	3	3	44	86.7	82.7
32	MLR2	1.59	1.59	1.59	1.59	2.09	3.59	5	188	63	5	5	93	89.7	82.1
33	MLR3	1.33	1.33	2.00	2.00	2.00	3.00	4	160	54	4	4	92	77.3	67.7
34	MLR4	1.33	2.00	2.00	2.00	2.00	2.33	3	141	47	3	3	29	90.1	82.8
35	MLR5	2.00	3.33	3.33	3.33	4.00	4.33	4	246	83	4	4	100	81.1	72.3
36	MLR6	1.67	1.67	1.67	1.67	2.00	3.00	3	154	52	3	3	97	74.7	70
37	MLR7	2.00	2.33	2.67	2.67	3.00	3.33	4	194	65	4	4	84	88.7	80.7
38	MLR8	1.33	1.67	1.67	1.67	2.00	2.33	3	144	48	3	3	67	83	79.7
39	MLR9	1.33	1.33	1.33	1.33	2.00	2.67	3	145	49	3	3	88	77.3	66.3
40	MLR10	2.00	2.00	2.00	2.00	2.00	3.00	4	178	60	5	5	94	87.1	79.3
41	MLR11	2.09	2.59	2.59	2.59	2.59	3.09	4	185	62	4	4	90	79	71.1
42	MLR12	2.00	2.00	2.33	2.33	2.33	2.67	3	161	54	3	3	58	83.3	73.3
43	MLR13	1.33	1.33	1.67	1.67	2.00	2.67	3	147	49	3	3	75	78.7	72
44	MLR14	2.00	2.33	2.33	2.33	2.67	3.67	4	193	65	4	4	89	73.5	67.6
45	MLR15	3.00	3.33	3.33	3.33	3.33	4.00	4	235	79	4	4	94	80.7	73.3
46	MLR16	1.67	2.00	2.00	2.00	2.00	3.00	3	161	54	3	3	97	77.3	67.3
47	MLR17	2.00	2.00	2.00	2.00	2.00	2.50	2	141	47	3	3	70	81.6	70.3
48	MLR18	1.33	1.67	1.67	1.67	2.00	2.67	3	149	50	3	3	75	71.3	66.3
Least significant difference									21.02	7.05		0.47	14.14	6.62	8.623
%Coefficient of variation									6.90	6.90		6.50	11.30	1	2.4

*=Significant difference at 5%; MLN Severity: was assessed based CIMMYT scale (0-1) where 0 = No MLN symptoms and 5 = Dead heart symptoms/ complete plant death; AUDPC: area under the disease progress curve calculated from the weekly MLN severity scores based on Wilcoxson *et al*., (1975) equation; ; rAUDPC: relative Area Under Disease Progress Curve calculated as a percentage of the AUDPC; % MLN incidence: percentage of the number of leaves with MLND infection; LSD=Least significant difference, CV= Coefficient of variation DTS= Days to 50% silking, DTP= Days to 50% Pollen shed

Table 7. Correlation among the traits across two seasons

Trait	AUDPC	DTP	DTS	FS	% DI	week1	week2	week3	week4	week5	week6	Week7
AUDPC	-											
DTP	0.43*	-										
DTS	0.47*	**0.90***	-									
FS	**0.85***	0.31*	0.35*	-								
%DI	**0.64***	0.11	0.16*	**0.64***	-							
week1	**0.51***	-0.09	-0.02	0.40*	0.42*	-						
week2	**0.57***	-0.11	-0.07	0.40*	0.38*	**0.73***	-					
week3	**0.54***	-0.23*	-0.20*	0.43*	0.40*	**0.64***	**0.84***	-				
week4	**0.59***	-0.16*	-0.14*	0.47*	0.45*	**0.60***	**0.77***	**0.90***	-			
week5	**0.66***	-0.09	-0.04	**0.53***	0.48*	**0.61***	**0.77***	**0.88***	**0.92***	-		
week6	**0.82***	0.20*	0.22*	**0.69***	**0.60***	**0.57***	**0.61***	**0.64***	**0.72***	**0.75***	-	
week7	**0.84***	0.36*	0.36*	**0.86***	**0.56***	0.44*	0.45*	0.46*	**0.54***	**0.60***	**0.81***	-

*=Significant difference at 5%; MLN Severity: was assessed based CIMMYT scale (0-1) where 0 = No MLN symptoms and 5 = Dead heart symptoms/ complete plant death; AUDPC: area under the disease progress curve calculated from the weekly MLN severity scores based on Wilcoxson et al. (1975) equation; % MLN incidence: percentage of the number of leaves with MLN infection; DTS= Days to 50% silking, DTP= Days to 50% Pollen shed

4. Discussion and Conclusion

Continuous efforts to identify resistant sources of MLN for introgression into the adapted maize genotypes are imperative in combating the sporadic nature of MLN causal agents especially in the face of climate change. The current research which involved assessing the response of assorted maize genotypes to the MLN disease has revealed the reaction of the genetically divergent maize genotypes. The plants exhibited clear mosaic symptoms on leaves, systemic in nature and which were indicative of susceptibility to MLN. Significant differences were reported among the genotypes with response to the different MLN assessment parameters. When a virus infects a plant, a signal transduction leads to physiological changes in the host plant implying that the genetic structure of the plants play a critical role in the infection and spread of viruses (Salaudeen and Aguguom, 2014). The significant and positive correlation coefficients noted between the MLN disease parameters validated their use in the assessment of the genotypes for response to MLN. Previous studies have identified susceptible and resistant genotypes by using the same parameters (Zambrano *et al*., 2013).

More than 50% of the genotypes screened in the two seasons were susceptible to the disease, validating the risk that MLN poses to maize production in Kenya and its food security status. These findings corroborate with reports by Gowda *et al.* (2015) on the high susceptibility of Kenyan maize germplasm to the MLN especially under artificial MLN infections. The MLN infection was induced by the artificial inoculation of SCMV and MCMV suggesting that the two viruses increased the viral load leading to the symptomatic effects which are both additive and lethal (Marçon *et al*., 1997). This study involved CIMMYT germplasm previously depicted as resistant in other studies. In this study, some lines still exhibited resistant responses namely MLN1, MLN15, MLN16, MLN17, MLN19, MLN18 and MLN12 which had low MLN severity ranging from 2-3 and also statistically low AUDPC, rAUDPC and % MLN incidence values. However, most of the other CIMMYT lines succumbed to the MLN disease.

Clear symptoms were observed among the susceptible MLN lines. Symptoms arise from interactions of the host with the virus and are as a result of compatible interaction which lead to the diversion of assimilates from the host plant to favour the virus cellular processes like its replication and multiplication (Revers *et al*., 1999). The resistance on the other hand could imply an incompatible interaction which could have been stimulated by the rapid necrosis at the foci of virus entry preventing its further spread and this could probably explain the partial resistance observed among some of the maize genotypes in this research work (Ronde *et al*., 2014). Previous studies have reported intense susceptibility to MLN in East Africa (EA) among the pre-commercial and commercial maize varieties (Jumbo *et al*., 2015; Semagn *et al*., 2015). It can be deduced that the use of germplasm labelled as resistant or moderately resistant should be done with caution and there is need for validation through repeat experiments over several seasons with reliable inoculations (CIMMYT, 2013; Gowda *et al*., 2015).

Among the farmer varieties, MLR-10, MLR-13 and MLR-17 MLR4, MLR6, MLR9, MLR18 and MLR16 were superior for MLN resistance These genotypes combined low AUDPC values which implies that the plant defense mechanism against the viruses could be mediated by resistance (R) genes which are observed as complete resistance or extreme resistance (ER) and that the virus replication could have been hindered or gone

undetectable among the infected cells (Ingvardsen *et al*., 2010). The farmer varieties are highly heterogeneous due to out-crossing within the farmers' fields. The presence of some resistance among these genotypes could offer the much-needed alternative to resource-constrained Kenyan farmers in combating the MLN threat.

This study also revealed that none of the genotypes was immune to the MLN disease across the two seasons of evaluation. However, some genotypes showed clear hypersensitive responses and necrotic symptoms. Previous studies by Zambrano *et al*. (2014) have reported presence of some resistance to multiple viruses in the family potyviridae. The presence of passive and active defense mechanisms hinders virus multiplication and spread influencing either the susceptibility or the resistance of germplasm (Zambrano *et al*., 2014).

The weekly MLN progression among the maize genotypes gave a great insight into the virus dynamics. Among the most susceptible genotypes, there was high AUDPC values coupled with high MLN scores as the weeks progressed. Revers *et al*. (1999) explained that the Potyviruses tend to follow the sink to source criteria in photo-assimilate partitioning. The plants viruses are known to gain access to the plant followed by the viral interaction with the host cells. This leads to the manipulation of the host cell pathways into viral factories (Sharma and Misra, 2011). Further on, the systemic infection of the viruses from the primary infection point and its invasion of the distal regions through the mesophyll into the bundle sheath cells, phloem parenchyma, and companion cells into phloem sieve elements is through passive translocation in the phloem leading to further infection and spread (Revers *et al*., 1999). However, among some genotypes, the symptoms plateaued while in others there was increased MLN severity with time. The MLN causal viruses cause systemic infections and the virus translocation from the point of inoculation depends on the cell-to-cell movement of its particles after the viral replication and establishment (Salaudeen and Aguguom, 2014). The resistant genotypes with low AUDPC values on the other hand could have had the inherent ability to retard the virus infection by inhibiting the movement of the virus inside the host cells (Gowda *et al*, 2015).

The superior maize genotypes identified in this research could serve as potential donors to improve the adapted maize varieties to combat the MLN threat in Kenya. However, the low frequency of resistance sources is an issue of great concern and this affects the nutritional and food security status of Kenya which is highly dependent on maize as a key staple food crop. Further evaluation of these elite genotypes for response to the singular viruses and establishment of the genetics of the MLN resistance will enhance their efficient utilization in breeding programs. Through genetic studies, the nature of the genes conditioning resistance to the MLN and its causal virus will help to elucidate further the viruses' dynamics.

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