The Response of Single Crosses to Inoculation with Maize Lethal Necrosis

Cheruiyot Susan[1], Oliver Kiplagat[1] and Philip Leley[2]

University of Eldoret, P.O Box 2666-30200 Kitale[1]
Email: cschumba@gmail.com; kiplagatoliver@yahoo.com

Kenya Agricultural and Livestock Research Organization,
P.O BOX 340-90100 Machakos, Kenya[2]
Email: pkleley@yahoo.com,

Abstract

Maize is by far the most important staple food commodity in many households in Kenya. Maize lethal Necrosis disease has in the recent past become a menace in hindering maize production in many regions of Kenya. A survey was carried out in Naivasha and Bomet to identify MLN resistant single cross genotypes growing under high disease pressure. MLN disease severity and MLN disease incidence was based on symptoms on the plant on a three weeks interval after planting until grain filling stage, plant stand count and yield. The causal pathogen was confirmed by diagnostic tests. There was a significant difference in resistance levels at p≤0.05 among the single cross varieties with respect to MLND. The scores ranged from 2.8- 3.9 in the two locations. Naivasha had higher disease scores with most severe symptoms in line SC-MLN-15-1 with a score of 4.0. Lines SC-MLN-15-6, SC-MLN-15-6, SC-MLN-15-7, SC-MLN-15-8, SC-MLN-15-15 and SC-MLN-15-37 showed moderate resistance. Lines SC-MLN-15-3 and SC-MLN-15-56 exhibited moderate resistance. High variability of MLN response was observed among the 120 maize genotypes indicating the exisistence of potential useful germplasm for improving MLN resistance for breeding programmes. It is concluded that MLN is still a persistent problem with high incidence associated with growing susceptible varieties, recycling hybrid seed and presence of alternative hosts for insect vectors; however resistant genes could be obtained from maize genotypes showing lower disease severity in these regions which have a high disease pressure which is a crucial strategy to ensure safe production of maize in the region.

Key words: Maize Lethal Necrosis, susceptible, resistant, varieties.

INTRODUCTION

Maize is an important staple food in Kenya as well as an important source of fodder in terms of dry matter Stover, thinning and green Stover for livestock (Murdoch *et al.,* 2013). Therefore, adequate amounts are required to feed the growing human population and an increasing need of maize for food and livestock feed. There has been a slight increase in national production level, but that is attributed to an increase in maize production area some of which are marginal unsuitable areas (Olwade and Smale 2012). The national yields however remain low, 1-2t/ ha against a potential of 6t/ha (Jaetzold *et al.,* 2006). This has been attributed to impoverished soils, unfavorable climatic conditions, pest and diseases (MoA, 2013). Global warming and its associated effects have changed weather patterns leading to erratic and unreliable distribution of rainfall resulting in drought. While most of these constraints have been generalized, this study sought to understand maize lethal necrosis disease as an important maize production constraint in Naivasha and Bomet counties.

MLN is one of the most damaging of all maize foliar diseases in many countries in the sub-Saharan region including Kenya. It was first observed in September 2011 at Longisa, Bomet County. Symptoms compatible with Maize Lethal Necrosis disease were noticed in 2012 in other areas of Kenya; Central, Nyanza and Rift valley provinces of Kenya. (Wangai *et al.,* 2012) MLN has remained a persistent constraint in maize production in Kenya (Magenya *et al.,* 2009) reducing both forage and grain yields (Murdoch *et al.,* 2003). Despite being an established and widely researched problem, susceptibility and high vulnerability of commercial cultivars remain a challenge leading to high losses.

Climate change especially the increased occurrence of drought conditions and high temperature favors and allows rapid multiplication of insects that transmit MLN. This has posed a challenge to scientists grappling with the disease (Wangai *et al.,* 2012). The outbreak of the disease poses a great danger to the agricultural sector in the country. To effectively manage MLN disease, there is need to identify MLN resistant varieties. This study was carried out to identify MLN resistant maize varieties grown under high disease pressure in Naivasha and Bomet counties. Therefore, this study focused on identifying the response of single cross varieties to MLN disease grown under high disease pressure.

MATERIALS AND METHOD

Experimental Site

There were two experimental sites. One under artificial inoculation at Naivasha {Latitude: 0°43.0002′ S Longitude: 36°26.1546′ E, 1915 m above sea level(asl) }and one under natural inoculation at Bomet[latitude 07°8565′ S, longitude 35°3391′ E, 2044 m above sea level (asl)].

Materials and Experimental Design

The trials comprised of 120 single cross hybrids set up in an incomplete block design in the two sites (Naivasha and Bomet). The single crosses were developed from resistant and susceptible inbred lines sources from CIMMYT AND KALRO. The trial in Naivasha was artificially inoculated twice.

Collection and Purification of MLN Viruses

The presence of SCMV and MCMV in the leaf samples was confirmed using ELISA and then transferred to H614 which is a susceptible hybrid. In different green houses, the leaf samples which were infected were collected , chopped, weighed and grinded using a blender in cold 0.1M potassium phosphate at buffer pH 7.0(ratio 1:10). The extract was then passed through a cheese cloth to remove any debris. The extract from the two viruses was then mixed and carborandum was added to decanted sap extract at the rate of 0.7 g/ l^{-1} of Inoculum and stirred to ensure even distribution of carborandum. The susceptible plants were inoculated at 3-4 leaf stage in the green house by rubbing sap onto the leaves with fingers. For Inoculum production, two separate sealed green houses for SCMV and MCMV were maintained. ELISA was then conducted 3 weeks pre- inoculation on leaf samples collected randomly from the different green houses to confirm purity of the Inoculum.

Artificial Field Inoculation

For even disease pressure in the fields the mixture of MLN viruses were mixed at a ratio of 4:1 and inoculated at 5^{th} - 6^{th} week post planting using a motorized pump (Solo 423 Mist Blower, 12 L capacity). The Inoculum spray was delivered at a rate of 120L/Ha^{-1} using an

open nozzle (2-inche diameter). Symptoms appeared 10 days after inoculation and ELISA was done to confirm presence of MLN viruses in the field trials.

Data Recording

Disease Severity

Data was collected on plant stand count and grain yield, data collection on disease severity was based on symptom observation in the susceptible control and rated as described by Gowda et al., 2015; 1- No symptoms on leaves, 2- Light symptoms on 20-40% leaf area, 3= moderate symptoms on 40-60% leaf area, 5= severe symptoms on 75% or more leaf area, plants severely stunted, drying/dead. Resistance was classified as follows;

1.0 –Symptomless, immune
1.2- 1.4 – Highly resistant
1.5-2.4– Resistant
2.5- 2.9– Moderately resistant
3.0- 5.0– Susceptible

MLN disease incidence was based on symptoms and diagnostic tests. Data on disease incidence and severity was recorded at 3week intervals after planting until the end of the grain filling period.

Disease Rating System

The disease rating system was visual and started two weeks post inoculation. It was conducted after every 14 days until 42 days post inoculation. Disease score was given on row basis. A minimum of 3 ratings were collected.

Data Analysis

The data was analyzed using Genstat (5^{th} edition).. Continuous variables which were found to observe normality test were reported as mean and its respective standard deviation. Regression analysis was used to find the correlation between dependent and independent variables. Association between continuous variable and categorical variables was done using Fisher's exact test. Kruskal- Wallis equality of rank test which is a Non-parametric statistical technique was used to test the equality of medians for the skewed continuous data across the two sites. Friedman statistical technique, which is a Non-parametric test, was used to find out any significant difference among the skewed continuous four related samples. The results were displayed using tables and graphs as shown below.

RESULTS

There were 240 (100%) maize plants in each site of the study and with each site having 120(50%) maize plants in rep 1and 120 (50%) maize plants in rep 2.

Site	Mean	SD	Sample (n)	P-value
Bomet	3.0769	0.336	240	0.05
Naivasha	3.533	0.325	240	

Analysis of variance for MLN score is presented in the tables 1 and 2. Mean squares for genotypes were highly significant. Genotype and environment interaction was also

significant. The severity scores for the inbred lines were variable and differences were significant (p=0.05) across environments.

The single crosses responded differently to infection with the MLN virus. The severity scores ranged from 2.8-3.9 across the two locations (table 4). Naivasha had a mean severity score of 3.6 while Bomet had a mean severity score of 3.1.

Naivasha had the highest disease scores with the most severe symptoms in lines: SC-MLN-15-1, SC-MLN-15-22, SC-MLN-15-77, SC-MLN-15-94 and SC-MLN-15-101with a score of 4.0. Lines with lowest disease scores in Naivasha included line SC-MLN-15-56 with a score of 2.5 and SC-MLN-15-6, SC-MLN-15-15, SC-MLN-15-29, SC-MLN-15-69 and SC-MLN-15-81 all with scores of 3.0 (Table 4).

In Bomet, Lines SC-MLN-15-1, SC-MLN-15-35 and lines SC-MLN-15-31 had the most severe symptoms with scores of 3.7, 3.7 and 3.8 respectively. Lines SC-MLN-15-3 and SC-MLN-15-23 had the lowest disease ratings with scores of 2.3 and 2.5 respectively. (Table 4).

DISCUSSION

High variability of MLN response was observed among the 120 maize genotypes indicating the exisisting and potentially useful germplasm for improving MLN resistance for breeding programmes.

Some of the inbreds were consistent in their disease reaction in the two environments notably; SC-MLN-15-15 and SC-MLN-15-6, while others showed varied response in the two sites: SC-MLN-15-77 (4, 3.2) and SC-MLN-15-94 (4, 2.9).

The difference in resistance levels among the lines bred for resistance may be attributed to different number of genes conditioning resistance or to the influence of genetic background.

Some lines with scores of ≤3.0 should be explored for probable use in transferring resistance to adapted but MLN susceptible backgrounds. It is important to explore and identify resistance emerging from different regions to improve diversity of populations for breeding disease resistance that target stability of resistance across regions.

REFERENCES

Jaetzold, R., H. Schmidt, B. Hornetz and C. Shisanya. 2006. Farm management handbook of Kenya. 2nd edition. Vol II. Part B. Central Kenya. Central Province. Ministry of Agriculture. Nairobi. Kenya.

Magenya, O.E.V., J. Mueke and C. Omwega. 2008. Significance and transmission of maize streak virus disease in Africa and options for management: A review. African Journal of Biotechnology 71: 4897-4910.

MoA. 2013. Food security assessment report. March 2013. Department of Crops Management. Ministry of Agriculture. Republic of Kenya.

Murdoch, A.J., J.M. Njuguna, B. Lukuyu, F. Musembi, D.M. Mwangi, J.M. Maina . 2003. Integrated pest management options to improve maize forage yield and quality for small-scale dairy farmers in central Kenya. Aspects of Applied Biology 70: 1-7.

Olwade, J. and M. Smale. 2012. Is older better? Maize hybrid change on household farms in Kenya. Selected paper prepared for presentation at the International Association of Agricultural Economists (IAAE). Triennial Conference, Foz do Iguacu, Brazil, 18-24 August, 2012.

Wangai, A.W., Redinbaugh, M.G., Kinyua, Z.M., Mahuku, G., Sheets, K., and Jeffers, D. 2012.First report of *Maize chlorotic mottle virus* and maize lethal necrosis in Kenya. Plant Dis.96: 1582.

TABLES/FIGURES

Table 1: Combined analysis of variance for MLN disease severity scores for genotypes screened at Bomet site

Variate: Disease score Grand mean: 3.076					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replicate	1	0.00521	0.00521	0.05	
Genotype	119	15.13253	0.12716	1.27	0.097
Residual	117	11.69987	0.10000		
Total	237	26.74290			
C.V	10.3				
LSD	0.6263				

Table 2: Combined analysis of variance for MLN disease severity scores for genotypes screened at Naivasha site

Variate: D_Score					
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REP stratum	1	0	0	0	
ENTRY	119	12.48	0.10	0.98	0.546
Residual	119	12.75	0.11		
Total	239	25.23			
C.V	9.3				
l.s.d.	0.6481				

Table 3: Combined analysis of variance for MLN disease severity scores for genotypes screened at Naivasha and Bomet in 2015

Source	df	ms	F	Prob > F
Model	2	12.4490233**	113.77	0.0000
Location	1	24.8975486**	227.54	0.0000
Rep	1	.00187314	0.02	0.8960
Residual	475	.109419704		
Total	477	.161158084		

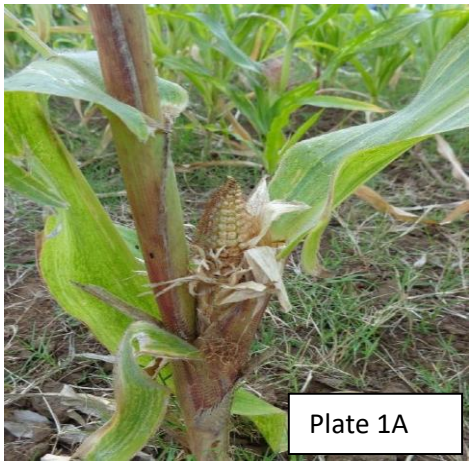
Table 4: Means of MLN scores in 120 inbreds screened in 2015 in two locations.

Genotype	Naivasha	Bomet	Across sites	Disease reaction
SC-MLN-15-1	4	3.7	3.9	S
SC-MLN-15-2	3.5	3	3.3	S
SC-MLN-15-3	3.3	2.3	2.8	MR
SC-MLN-15-4	3.5	3.2	3.4	S
SC-MLN-15-5	3.8	2.7	3.3	S
SC-MLN-15-6	3	3	3	S
SC-MLN-15-7	3.8	3.1	3.1	S
SC-MLN-15-8	3	2.9	3	S
SC-MLN-15-9	3.8	3.1	3.5	S
SC-MLN-15-10	3.8	3	3.4	S
SC-MLN-15-11	3.8	3.5	3.7	S
SC-MLN-15-12	3.8	3.1	3.5	S
SC-MLN-15-13	3.8	3.1	3.5	S
SC-MLN-15-14	3.8	2.8	3.3	S
SC-MLN-15-15	3	2.9	3	S
SC-MLN-15-16	3.3	3	3.2	S
SC-MLN-15-17	3.6	3.1	3.4	S
SC-MLN-15-18	3.8	2.9	3.4	S
SC-MLN-15-19	3.3	3	3.2	S
SC-MLN-15-20	3.3	3.4	3.4	S
SC-MLN-15-21	3.5	2.8	3.2	S
SC-MLN-15-22	4	3.1	3.6	S
SC-MLN-15-23	3.8	2.5	3.2	S
SC-MLN-15-24	3.8	2.9	3.4	S
SC-MLN-15-25	3.5	2.9	3.2	S
SC-MLN-15-26	3	3.5	3.3	S
SC-MLN-15-27	3.5	3.4	3.5	S
SC-MLN-15-28	3.8	3.2	3.5	S
SC-MLN-15-29	3.8	2.7	3.3	S
SC-MLN-15-30	3.5	3.3	3.4	S
SC-MLN-15-31	3.5	3.8	3.7	S
SC-MLN-15-32	3.8	3.3	3.6	S
SC-MLN-15-33	3.5	3	3.3	S
SC-MLN-15-34	3.8	3	3.4	S
SC-MLN-15-35	3.5	3.7	3.6	S
SC-MLN-15-36	3.8	2.9	3.4	S
SC-MLN-15-37	3.3	2.9	3.1	S
SC-MLN-15-38	3.5	3.1	3.3	S
SC-MLN-15-39	3.8	3.3	3.6	S
SC-MLN-15-40	3.5	3.3	3.4	S
SC-MLN-15-41	3.8	2.9	3.4	S
SC-MLN-15-42	3.5	3.3	3.4	S
SC-MLN-15-43	3.5	2.8	3.2	S
SC-MLN-15-44	3.8	3.3	3.6	S
SC-MLN-15-45	3.5	3	3.3	S
SC-MLN-15-46	3.5	2.8	3.2	S
SC-MLN-15-47	3.5	3.2	3.3	S
SC-MLN-15-48	3.5	3	3.3	S

SC-MLN-15-49	3.3	2.8	3.1	S
SC-MLN-15-50	3.8	3.5	3.7	S
SC-MLN-15-51	3.8	3.2	3.5	S
SC-MLN-15-52	3.5	3	3.3	S
SC-MLN-15-53	3.8	2.8	3.3	S
SC-MLN-15-54	3.8	2.8	3.3	S
SC-MLN-15-55	3.5	2.9	3.2	S
SC-MLN-15-56	2.5	3.2	2.9	MR
SC-MLN-15-57	3.5	3.3	3.4	S
SC-MLN-15-58	3.3	3.1	3.2	S
SC-MLN-15-59	3.5	3.7	3.6	S
SC-MLN-15-60	3.8	3	3.4	S
SC-MLN-15-61	3.8	2.8	3.3	S
SC-MLN-15-62	3.5	2.9	3.2	S
SC-MLN-15-63	3.5	2.7	3.1	S
SC-MLN-15-64	3.5	3	3.3	S
SC-MLN-15-65	3	3.4	3.2	S
SC-MLN-15-66	3.5	3.2	3.4	S
SC-MLN-15-67	3.5	2.8	3.2	S
SC-MLN-15-68	3.5	2.9	3.2	S
SC-MLN-15-69	3	2.9	3	S
SC-MLN-15-70	3.5	3.3	3.4	S
SC-MLN-15-71	3.8	3	3.4	S
SC-MLN-15-72	3.3	3.5	3.4	S
SC-MLN-15-73	3.3	3	3.4	S
SC-MLN-15-74	3.5	2.9	3.2	S
SC-MLN-15-75	3.8	3.2	3.5	S
SC-MLN-15-76	3.8	3.5	3.7	S
SC-MLN-15-77	4	3.2	3.6	S
SC-MLN-15-78	3.5	3.1	3.3	S
SC-MLN-15-79	3.5	3.2	3.4	S
SC-MLN-15-80	3.5	2.9	3.2	S
SC-MLN-15-81	3	2.9	3	S
SC-MLN-15-82	3.5	3	3.3	S
SC-MLN-15-83	3.5	3.3	3.4	S
SC-MLN-15-84	3.8	3.5	3.7	S
SC-MLN-15-85	3.5	2.9	3.2	S
SC-MLN-15-86	3.8	3.5	3.7	S
SC-MLN-15-87	3.8	3.4	3.6	S
SC-MLN-15-88	3.8	3	3.4	S
SC-MLN-15-89	3.5	2.9	3.2	S
SC-MLN-15-90	3.5	3	3.4	S
SC-MLN-15-91	3.8	3.3	3.6	S
SC-MLN-15-92	3.3	3.2	3.3	S
SC-MLN-15-93	3.8	3.4	3.6	S
SC-MLN-15-94	4	2.9	3.5	S
SC-MLN-15-95	3.8	3.2	3.5	S
SC-MLN-15-96	3.3	2.9	3.1	S
SC-MLN-15-97	3.5	3.3	3.4	S
SC-MLN-15-98	3.5	2.9	3.2	S

SC-MLN-15-99	3.5	2.8	3.2	S
SC-MLN-15-100	3.5	2.9	3.2	S
SC-MLN-15-101	4	3.3	3.7	S
SC-MLN-15-102	3.5	2.9	3.2	S
SC-MLN-15-103	3.3	3	3.2	S
SC-MLN-15-104	3.5	3.3	3.4	S
SC-MLN-15-105	3.8	3.3	3.6	S
SC-MLN-15-106	3.5	2.7	3.1	S
SC-MLN-15-107	3.3	3	3.2	S
SC-MLN-15-108	3.5	3.2	3.4	S
SC-MLN-15-109	3.8	3.3	3.6	S
SC-MLN-15-110	3.8	3	3.4	S
SC-MLN-15-111	3.3	3.1	3.2	S
SC-MLN-15-112(check-1)	3.5	2.9	3.2	S
SC-MLN-15-113(check 2)	3.5	3.1	3.3	S
SC-MLN-15-114(check 3)	3.8	3.1	3.5	S
SC-MLN-15-115(check4)	3.5	3.4	3.5	S
SC-MLN-15-116(check5)	3.5	3.2	3.4	S
SC-MLN-15-117(check6)	3.5	3.1	3.3	S
SC-MLN-15-118(check7)	3.5	2.9	3.2	S
SC-MLN-15-119(check 8)	3.5	3.3	3.4	S
SC-MLN-15-120(check9)	3.5	3.2	3.4	S
Mean	3.56	3.09	3.35	
Lsd(0.05)	1.10	0.95	1.03	
CV (%)	0.065	8.23	5.714	

Figure 1: Symptoms of maize lethal necrosis disease



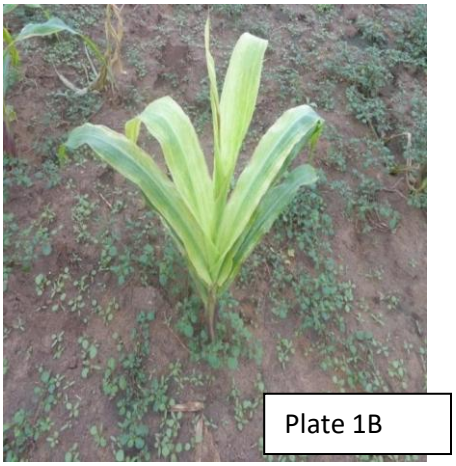


Plate 1: Symptoms of MLN disease in Naivasha and Bomet (Plate 1A) Chlorosis on leaves, (Plate 1B) Small cobs with little or no grains (Plate 1C) Necrosis followed by plant death, (Plate 1D) Mottling and necrosis start from margins to the mid-rib; Photos by Susan cheruiyot.