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The effect of Potyvirus resistance loci from the maize inbred line Oh1VI on development of maize lethal necrosis (MLN)

Victoria B. Bulegeya[1]*; Mark W. Jones[2]; Tryphone G. Muhamba[3]; Biswanath Das[4]; Peter R. Thomison[5]; David M. Francis[6]; Margaret. G. Redinbaugh[7]

1- Tanzania Agriculture Research Institute (TARI) – Dakawa Center, P.O.Box 1892, Morogoro, Tanzania.
2- United States Department of Agriculture–Agricultural Research Service (USDA-ARS), Corn, Wheat and Soybean Research, Wooster, OH 44691, USA;
3- Department of Crop Science and Horticulture, Sokoine University of Agriculture (SUA), P.O.Box 3005, Morogoro, Tanzania
4- International Maize and Wheat Improvement Center (CIMMYT), P.O.Box 1041, Village Market, Nairobi 00621, Kenya
5- Department of Horticulture and Crop Science, The Ohio State University, 2021 Coffey Rd, Columbus, OH 43210, USA.
6- Department of Horticulture and Crop Science, The Ohio State University-Ohio Agriculture Research and Development Center (OARDC), Wooster, OH 44691, USA
7- USDA-ARS, Corn, Wheat and Soybean Research, Department of Plant Pathology, The Ohio State University, Wooster, OH 44691, USA.

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***Corresponding Author**
Victoria Bulegeya
E-mail: *victoriabulegeya@rocketmail.com*

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ABSTRACT

Maize lethal necrosis (MLN), a viral disease currently affecting corn in East and Central Africa is caused by a combined infection of Maize chlorotic mottle virus (MCMV) and any maize infecting potyvirus. Most of African maize germplasm is susceptible to the disease and there are no known sources of resistance. Recombinant inbred lines (RIL) derived from Oh1VI, a line known for multi-virus resistance with different QTL for potyvirus resistance on chromosome 3, 6 and 10 were selected and screened against MLN under artificial inoculation and natural infestation. Differences were observed among genotypes and QTL groups at P=0.05 in all experiments except under field inoculation. Genotypes with QTL combination of 3, 6 and 10 had at least 20% reduction in MLN symptoms compared to a susceptible check. These results provide useful baseline information on utilization of potyvirus resistance genes for MLN resistance and control in Sub Saharan Africa.

1. INTRODUCTION

Maize lethal necrosis (MLN) is a disease currently affecting corn (*Zea mays*) production in East and Central Africa (Mahuku et al., 2015a, 2015b; Wangai et al., 2012; Adams et al., 2013, 2014; Lukanda et al., 2014). MLN is caused by combined infection of Maize chlorotic mottle virus (MCMV) and any maize infecting virus in the *Potyviridae* family such as Wheat streak mosaic virus (WSMV), Maize dwarf mosaic virus (MDMV) and Sugarcane mosaic virus (SCMV) (Niblett & Claflin 1978; Uyemoto et al., 1980). In East Africa the primary cause of the disease is co- infection with Maize chlorotic mottle virus and Sugarcane mosaic virus (Wangai et al., 2012; Adams et al., 2014; Lukanda et al., 2014; Mahuku et al., 2015).

A survey carried out in East African countries to study the distribution of MLN causing viruses suggested up to 94% incidence in randomly selected symptomatic plants (Mahuku et al., 2015). Tanzanian samples collected at Arusha and Mwanza had 60% to 69% incidence and both viruses were detected (Mahuku et al., 2015). The survey indicated wide distribution and high prevalence of MLN viruses in East and Central Africa.

MLN causes chlorotic mottling from the plant base, leaf necrosis from the margins to the midrib, stunted plant growth, premature death, male sterility and failure to tassel, malformed ears or lack of ear formation, and rotten or small cobs with little or no grain fill (Niblett & Claflin, 1978; Wangai et al., 2012). The magnitude of yield loss associated with the disease makes developing cultivars with disease resistance crucial. In Kenya, MLN caused an estimated loss of $187 million equivalent to $364/ton in 2012 (De Groote et al., 2016). Farmers in MLN areas have experienced a significant decrease in yield since MLN was first reported in 2010 (Makone et al., 2014).

Potyviruses are endemic to East Africa and were observed to cause crop loss of 18% to 46% (Louie, 1980). The introduction of MCMV and co-infection of maize with the endemic potyviruses to cause MLN represents a new threat to maize production in East African countries (Wangai et al., 2012). There is a need for identification of MLN resistance sources, mapping of genomic regions with MLN resistance and intogression of resistance genes into widely used susceptible inbred lines and hybrids in East Africa (Semagn et al., 2015). The study evaluated Recombinant Inbred Lines (RIL) with potyvirus resistance QTL in disease hotspots in Tanzania and under high disease pressure through artificial inoculation in the growth chamber and field. The RIL population is derived from multi-virus resistant parent Oh1VI and a susceptible parent Oh 28. The population was genotypically analyzed for potyvirus resistance and QTL for potyvirus resistance were mapped to chromosome 3, 6 and 10 (Zambrano et al., 2014). Selected lines with combinations of the QTL were used to analyse the influence of potyvirus resistance in MLN control. The study aimed to fill the knowledge gap concerning the influence of potyvirus resistance QTL for the control of MLN and the suitability of temperate lines in managing MLN in Africa.

2. MATERIALS AND METHODS

2.1 Plant materials

Inbred lines selected from a Recombinant Inbred line (RIL) population derived from a multi-virus resistant parent Oh1VI and susceptible parent Oh28 were used for the study. The RIL population was generated by the Corn, Soybean and Wheat Quality Research Unit (CSWQRU) at the Ohio Agricultural Research and Development Centre (OARDC). The RIL population was previously genotyped with 768 single nucleotide polymorphism (SNP) markers and QTL for potyvirus resistance (Zambrano et al., 2014). Selections were based on molecular markers flanking QTL for potyvirus resistance on chromosomes 3, 6 and 10 alone and in all possible combinations. Flanking markers PHM13823-7 and PZA00667-1 were used to select for chromosome 3 QTL, markers PHM15961-13 and PZA00540-3 selected chromosome 6 QTL and flanking markers PHM1812-32 and PHM15868-5 selected chromosome 10. Five independently chosen lines represented one individual QTL or a combination QTL from chromosome 3, 6 and 10. Lines 80231, 80229, 80209, 80221 and 80196 had allele for resistance on chromosome 3, 6 and 10 forming a treatment group of 3_ 6_10.

Genotypes were planted for evaluation in a growth chamber at the Department of Plant Pathology, Ohio State University/OARDC, Wooster, Ohio, May to July 2015 and at the CYMMIT – KALRO MLN Screening Facility, Naivasha, Kenya in December 2015 to March 2016. In natural infection trials treatments were planted for evaluation in fields at Babati – Manyara (latitude: -4.20963602, longitude: 35.73990726, elevation: 1378m) and Mlangalini – Arusha (latitude: -3.3666700, longitude 36.6833300, elevation 1415m), Tanzania during the rain seasons of 2015 and 2016.

In all experiments except for the field inoculation and 1st natural infestation experiment, both parents were included as resistant and susceptible controls and to provide baseline information on disease incidence and severity on each experiment. Control lines 80066 and 80293 from Oh1VI RIL population which lack resistance alleles on all three chromosomes were included in a growth chamber experiment and CML444 and entry73 tropical lines from CIMMYT were included as controls in a field inoculation experiment as susceptible local controls and local checks CML144, CML197, CML442, CML395, KS23-6 and KS23-5 were used in a natural infection experiment.

2.2 Viral inoculum sources

The isolates of SCMV and MCMV used for a growth chamber experiment were maintained by the USDA, CWSQRU. The SCMV-OH isolate was collected from Ohio (Louie, 1986) and the MCMV-KS isolate was collected from Kansas (Niblett & Claflin, 1978). The sequence of MCMV-KS is 96%-97% identical to the East African isolate which is 98%-99% identical to isolates from China (Mahuku et al., 2015). The SCMV-OH isolate was maintained by serial mechanical transmission to a susceptible maize line, and the MCMV-KS isolate was stored frozen and in liquid nitrogen and transmitted to the susceptible line Oh28 as a source of inoculum. Presence of the viruses in symptomatic plants was confirmed by tissue blot immunoassay as previously described (Jones et al., 2011). Inoculum made from a mixture of infected leaf tissues for both viruses was prepared in a combination of 1:4 MCMV to SCMV to attain uniform MLN pressure. Inoculum was prepared by grinding symptomatic leaf tissues in a 0.1 M potassium phosphate in 1:10 dilution ratio (1 gram of tissue to 10 milliliters of the 0.1M, 7.0 pH potassium phosphate buffers) using mortar and pestle. Carborundum (0.02 g/ml) was added as an abrasive agent. The prepared inoculum was rub inoculated to leaves of 14 days old seedlings (Jones et al., 2007). There were two inoculations per experiment with the second inoculation applied two days after the first to ensure successful infection. Plants were transferred to a growth chamber with a 25-21°C (day-night), 75% relative humidity, 532 μmol light intensity (microeinsteins) and a 12 hr photoperiod.

In a field inoculation experiment the inoculum was prepared following the protocol used at the MLN screening facility at Naivasha under CIMMYT and KARLO using East African isolates of SCMV and MCMV maintained through serial transmission to susceptible maize (Gowda et al., 2015). The inoculum was made from a mixture of symptomatic tissues with individual infection of SCMV and MCMV in a combination of 4:1 ratio respectively. The inoculum was prepared by harvesting the plants infected with SCMV and MCMV separately, and then leaves were chopped, weighed and blended in 0.1M potassium phosphate buffer with 1:20 dilution ratio (leaf material: buffer) at a pH of 7.0 and sieved to remove plant debris. The inoculum was mixed in a larger tank and Carborundum 1g/liter was added. Field inoculation was done using a motorized mist blower (Solo423 MistBlower, 11 liter capacity). The inoculum was delivered at a pressure of 10 kg/cm^2 with a 2-inch nozzle. Inoculation was carried out at the 4-6 leaf stage and repeated after one week.

2.3 Experimental design

All experiments were established following the alpha lattice design. Except for trial 4 under natural infection all experiments were arranged in an alpha lattice design of 42 treatments in 3 replications; each replication consisted of 6 blocks with 7 treatments each. Trial 4 had a total of 40 treatments and each replication had 4 blocks of 10 treatments. Each treatment was planted in a row of 5m with intra-row spacing of 25 cm and inter-row spacing 75 cm. All trials were planted under rainfed conditions; irrigation was supplementary in non-rain days and Diammonium phosphate (DAP) was added at planting and UREA as a top dressing to supplement nitrogen and phosphorus sources using local recommended rates.

2.4 Data collection

For the growth chamber experiment plants were evaluated for disease development beginning 7 days post second inoculation and rating continued every four days until 23 days post inoculation. For the field inoculation experiment disease rating was done 2 weeks post inoculation continuing every 7 days until 42 days post inoculation and for natural infection disease severity ratings were initially performed every seven days and then extended to 14 days covering a total of 56 days. Disease was scored on a scale of 1 to 5 as follows: 1 = no visible MLN symptoms, 2 = fine chlorotic streaks mostly on older leaves, 3 = chlorotic mottling throughout the plant, 4 = excessive chlorotic mottling on lower leaves and necrosis of newly emerging leaves (dead heart), and 5 = complete plant necrosis (Gowda et al, 2015). Severity scores collected were used to generate area under the disease progress curve (AUDPC) values. The equation for AUDPC is

$$\sum_{i}^{n-1}\left[\frac{Yi+Y(i+1)}{2}\right]*\left[\left((ti+1)-ti\right)\right]$$

where; Yi is disease assessment (score), at the ith observation, ti is the time of observation (days) at the ith observation and n is the total number of observation. First scores, last scores mean scores and AUDPC values were used to test for differences among treatments.

2.4 Data analysis

Analysis was done using the R package version 3.1.1(R Development Core team, 2014). The Agricolae package version 1.2-3 (de Mendiburu, 2010) was used to test for differences among treatments in measured parameters. The experimental model for the alpha lattice was Yij = μ (Mean effect) + Ri (Replicate) +Tj (Treatment effect) + βi (Incomplete Block effect) +Ɛij (Intra –block error effect). The *PIBl.test* function was used for the partial incomplete block design to correct for incomplete block effects (de Mendiburu, 2010). A two-tiered analysis was conducted in which the adjusted means from the alpha-lattice were then used to test the null hypothesis that there are no differences between higher order QTL treatments when comparing 3, 6, and 10 alone; 3 and 6, 3 and 10, 6 and 10, in combinations; and 3, 6 and 10 together. The later model was then tested using a general linear model in the R core package version 3.1.1(R Development Core team, 2014). Since different checks were used in different experiments, each

experiment was analysed differently and all the treatments were normalized to a susceptible check Oh28.

3. RESULTS

3.1 Response of genotypes to natural and artificial infestation of MLN

In the growth chamber, there was significant variation among genotypes and among different combinations of potyvirus resistance QTL to MLN inoculation. Genotypes with combinations of resistance QTL groups from chromosome 3, 6 and 10 developed less disease symptoms compared to genotypes with single resistance QTL (Table 1).

Under field inoculation no significant differences in MLN symptoms expression was observed between individual RIL genotypes and controls or for QTL groups. Field ratings were conducted over an extended 42 days period, which may have affected our ability to discern differences. The analysis based on QTL groups indicates that genotypes with QTL combinations of 3 + 6 + 10 has significantly lower means and AUDPC scores compared to other genotype groups. However, the adjusted means for first scores and last scores were not significantly different in the field environment (Table 1).

There was no significant variation among genotypes with different QTL combinations in trial 1 set at Mlangalini, Arusha, Tanzania presumably due to low incidence. Significant variation (P = 0.05) among QTL groups was observed in 3 experiments (trials 2 through 4) set at Krishna seed farm and KIRU-6 village at Babati, Manyara, Tanzania in the first scores, last scores, mean scores and AUDPC (Table 1).

Table 1. Importance of specific QTL and QTL combinations in response to MLN infection under natural infestation and artificial inoculation.

Environment [h]	QTL groups [i]	Severity score [j]			
		FIRST SCORE	**LAST SCORE**	**MEAN SEVERITY**	**AUDPC** [k]
Growth chamber (OHIO- US)	Oh28[l]	3.06a	4.97a	4.23a	52.74a
	80066[m]	3.21a	4.70ab	4.08a	46.88ab
	80293[n]	2.76a	4.33abc	3.62ab	43.16abc
	10	1.95b	4.04abc	3.09bc	36.63bcd
	6	1.91b	4.00abc	3.05bc	34.75bcd
	10_6	1.76bc	3.45bc	2.80bc	34.32cd
	3	1.72bc	3.26bc	2.66cd	29.58de
	3_6	1.43cd	2.90c	2.28d	26.51e
	3_6_10	1.21d	3.08c	2.27d	25.61ef
	3_10	1.27d	3.16c	2.17de	25.57ef
	Oh1V1[o]	1.23d	1.38d	1.35e	13.32f
P-values		2.2×10^{-8}***	0.001***	3.1×10^{-6}***	1.173×10^{-3}***
Field inoculation (KENYA)	Oh28[l]	3.18a	4.33ab	4.46a	96.20a
	Entry73[p]	2.82a	5.00a	4.36a	94.64a
	3_10	2.86a	4.68a	4.15a	89.11a
	83649[q]	2.80a	4.50ab	4.14ab	88.74ab
	6	2.90a	4.60a	4.09ab	88.32ab
	CML444[r]	2.80a	5.00a	4.11ab	87.98ab
	3_6	2.86a	4.89a	4.00ab	85.83ab
	3	2.96a	4.50ab	4.00ab	85.74ab
	10_6	2.76a	4.63a	3.97ab	85.31ab
	10	2.86a	4.90a	3.99ab	84.81ab
	3_6_10	2.67a	4.07ab	3.62b	78.00b
P- values		0.601 ns	0.601 ns	0.663 ns	0.651 ns
Natural infestation TRIAL 1(TANZANIA)	Oh28[l]	2.33a	2.33a	2.08a	41.73a
	3_10	1.75ab	1.40b	1.63ab	34.44ab
	83649[q]	1.62ab	1.67ab	1.90ab	41.80a
	10	1.54ab	1.67ab	1.71ab	36.74a
	3_6_10	1.51ab	1.60ab	1.74ab	37.87a
	3_6	1.50ab	1.44ab	1.61ab	34.78ab
	6	1.50ab	1.38b	1.63ab	35.51a
	10_6	1.39b	1.27b	1.57ab	34.79ab
	3	1.32b	1.12b	1.36b	29.17b
	Pannar[s]	1.00b	1.00b	1.17b	25.67b
	sc-627[t]	1.00b	1.00b	1.17b	25.67b
P-values		0.03*	0.12 ns	0.002**	0.009**

Natural infestation TRIAL 2 (TANZANIA)	Oh28 [d]	1.67a	3.5a	2.46a	101.5a
	CML197	1.50ab	3.33ab	2.25ab	92.17ab
	6	1.41ab	3.07bc	2.20b	91.73ab
	10	1.43ab	3.03bcd	2.19b	91.35ab
	3	1.34b	2.93bcd	2.12bc	89.04b
	10_6	1.40ab	3.03bcd	2.13b	88.55b
	3_6	1.33b	2.81de	2.09bc	88.17b
	3_10	1.37ab	2.87cd	2.09bc	87.42b
	CML144	1.50ab	2.83cde	2.08bc	86.33b
	3_6_10	1.31bc	2.60e	1.99c	84.22b
	Oh1V1[b]	1.00c	2.17f	1.58d	66.5b
P-values		0.02^{**}	0.001^{***}	$2.07x10^{-5***}$	$9.8x10^{-5***}$
Natural infestation TRIAL 3 (TANZANIA)	Oh28[l]	2.00a	4.00a	3.04a	128.3a
	10	1.97a	3.77a	2.92a	123.3a
	10_6	1.79ab	3.68ab	2.83a	120.0a
	6	1.74ab	3.64ab	2.78a	117.8a
	CML197[y]	1.34ab	4.00a	2.83a	117.8ab
	3	1.80ab	3.58b	2.74a	116.0ab
	CML144[z]	1.50bc	3.00cd	2.42bc	103.8bc
	3_6	1.58bc	3.18cd	2.43b	102.6c
	3_10	1.40c	3.29c	2.38bc	100.6c
	3_6_10	1.39c	2.98d	1.22c	93.86c
	Oh1V1[o]	1.33c	2.83d	1.17c	92.17c
P – values		$1.12x10^{-5***}$	$2.43x10^{-5***}$	$8.3x10^{-10***}$	$3.87x10^{-9***}$
Natural infestation TRIAL 4 (TANZANIA)	Oh28[l]	2.34a	3.83a	2.91a	120.0a
	CML442[u]	2.35a	3.50bc	2.88ab	120.2a
	CML395[v]	2.16ab	3.67bc	2.83abc	117.7ab
	10	2.06ab	3.50bc	2.77abc	116.0ab
	6_10	2.07ab	3.50bc	2.74bcd	114.5abc
	6	1.99ab	3.43cd	2.73cd	114.9ab
	3	2.05ab	3.43cd	2.70cd	112.6bc
	3_6	1.86bcd	3.47bcd	2.66de	111.5cd
	3_10	1.95abc	3.33de	2.50e	108.8de
	3_6_10	1.73cd	3.23e	2.50f	105.3ef
	KS523-6[w]	1.66cd	3.33de	2.41fg	100.2fg
	Oh1V1[o]	1.50de	3.33de	2.34g	97.05gh
	KS523-5[x]	0.98e	2.83f	2.08h	89.73h
P-values		$2.02x10^{-5***}$	0.001***	$1.2x10^{-8***}$	$1.92x10^{-7***}$

[h] Location with different mode of infection where maize genotypes were tested for resistance to MLN
[i] Groups of maize genotypes with Potyvirus resistance QTL on chromosome 3, 6 and 10 alone or in a combination of 2 and 3 QTL group.
[j] Severity scores collected at different time points under artificial inoculation and natural infestation.
[k] Area under disease progress curve (AUDPC) values calculate from disease severity scores at different time points.
[l] A susceptible parent
[m] Susceptible checks from Oh1VI RIL population with no resistance QTL from 3, 6 and 10
[n] Susceptible checks from Oh1VI RIL population with no resistance QTL from 3, 6 and 10
[o] A resistant parent
[p] A tropical line from CYMMIT susceptible to MLN
[q] A susceptible checks from a Oh1VI RIL population with no resistance QTL from 3, 6 and 10
[r] A tropical line from CYMMIT susceptible to MLN
[s] A local check, commercial hybrids used by farmers in Tanzania
[t] A local check, commercial hybrids used by farmers in Tanzania
[u] A tropical line from CYMMIT susceptible to MLN
[v] A tropical line from CYMMIT susceptible to MLN
[w] A Kansas line with resistance to MLN
[x] A Kansas line with resistance to MLN
[y] A tropical line from CYMMIT susceptible to MLN
[z] A tropical line from CYMMIT susceptible to MLN

3.2 Importance of potyvirus resistance QTL interaction for MLN control

The analysis indicated differences in disease development for germplasm with potyvirus resistance QTL compared to a susceptible control Oh28. In general, genotypes with a combination of three QTL from chromosomes 3, 6, and 10 performed the best across experiments, reducing disease severity by an average of 20% (Table 2). Also, combinations of 2 QTL (3 + 10 and 3 + 6) developed less MLN symptoms compared to genotypes with a single resistance QTL sources. These results indicate a role for QTL interaction in MLN control.

Table 2. Response of genotypes with specific QTL and QTL combinations to MLN infections normalized to a susceptible parent

QTL group[q]	MEAN[r]	LSD GROUP[s]
CML 442[t]	1.002	a
Oh 28[u]	1	a
CML395[v]	0.9808	a
10	0.927	ab
CML197[w]	0.9131	abc
6	0.9076	abc
6_10	0.8989	abc
3_6	0.8577	bcd
3	0.8547	bcd
3_10	0.8443	cd
3_6_10	0.8366	cd
KS523-6[x]	0.835	cde
KS523-5[y]	0.7478	def
Oh1V1[z]	0.7274	ef
P- value = 6.148e-10***		
Alpha level = 0.05		
Critical value = 2.04		

[q] Groups of maize genotypes with Potyvirus resistance QTL on chromosome 3, 6 and 10 alone or in a combination of 2 and 3 QTL group.
[r] Average severity scores collected at different time points under artificial inoculation and natural infestation.
[s] Least significant difference group in response average severity scores
[t] A tropical line from CYMMIT susceptible to MLN
[u] A susceptible parent
[v] A tropical line from CYMMIT susceptible to MLN
[w] A tropical line from CYMMIT susceptible to MLN
[x] A Kansas line with resistance to MLN
[y] A Kansas line with resistance to MLN
[z] A susceptible parent
.

3.3 Agronomic performance of genotypes under natural infection of MLN

Data on agronomic performance among genotypes shows a clear difference between RIL, QTL groups and local checks adapted to a tropical environment. Agronomic data were not collected from trials 3 and 4 because these experiments did not reach reproductive maturity. In parameters such as emergency%, days to flowering and yield there is a significant difference between RIL genotype and genotype groups in trial 1 and trial 2 (Table 3). In both trials the difference is seen with treatments and local checks since local checks were adapted hence they outweigh genotypes under study.

Table 3. Agronomic performance of genotypes with potyvirus resistance under natural MLN infection at MLN hotspot in Arusha and Babati.

	QTL group[s]	Emergence (%)	Flowering date (days)		Yield/ear (Kg)	Ear rot
			Anthesis	Silking		
Trial 1	Pannar[t]	93.33	69.00b	72.00b	0.1a	0.18b
	83649[u]	83.29	76.94a	80.00a	0.08a	0.05c
	3_6	73.45	74.11a	78.86a	0.05b	0.05c
	10	72.83	75.90a	80.00a	0.05b	0.05c
	3_10	68.58	74.43a	78.80a	0.05b	0.05c
	6	66.81	73.63ab	78.87a	0.05b	0.04c
	3_6_10	62.51	74.31a	79.17a	0.05b	0.04c
	3	62.51	74.14a	78.39a	0.04b	0.04c
	Oh28[v]	60.98	75.37a	79.56a	0.04b	0.05c
	10_6	52.02	74.18a	79.17a	0.03b	0.04c
	Sc-627[w]	28.33	73.33ab	77.33ab	0.03b	0.30a
P-values		$2.87\times10^{-8***}$	$7.58\times10^{-3***}$	$7.88\times10^{-6***}$	$2.2\times10^{-16***}$	0.05* S
Trial 2	CML197[x]	18.33c	64.33a	67.67a	0.1a	2.00c
	CML144[y]	45.00abc	62.00ab	65.67ab	0.08a	1.67c
	3_6	57.49a	57.20c	61.73bc	0.05b	3.83ab
	10_6	58.16a	57.04c	61.03c	0.05b	4.60a
	3_10	40.44bc	57.45c	61.42bc	0.05b	4.60a
	3_6_10	41.41bc	56.84c	61.24bc	0.05b	2.06a
	10	49.93ab	57.99c	61.78bc	0.05b	3.87ab
	6	47.76ab	58.42bc	62.67bc	0.04b	2.86bc
	3	42.54bc	56.84c	60.73c	0.04b	4.00ab
	Oh1VI[z]	25.00bc	58.33bc	61.67bc	0.03b	0.67c
	Oh28[v]	51.67ab	59.33abc	61.00c	0.03b	6.33a
P-values		0.002***	$8.5\times10^{-3***}$	0.002***	$9.9\times10^{-6***}$	0.027***

[s] Groups of maize genotypes with Potyvirus resistance QTL on chromosome 3, 6 and 10 alone or in a combination of 2 and 3 QTL group.
[t] A local check, commercial hybrids used by farmers in Tanzania
[u] Susceptible checks from Oh1VI RIL population with no resistance QTL from 3, 6 and 10
[v] A susceptible parent
[w] A local check, commercial hybrids used by farmers in Tanzania
[x] A tropical line from CYMMIT susceptible to MLN
[y] A tropical line from CYMMIT susceptible to MLN
[z] A resistant parent

4. DISCUSSION

The study aimed to determine which of the three potyvirus resistance QTL on chromosome 3, 6 and 10 might provide protection against MLN. No genotypes were unaffected by MLN, signifying that the QTL under study were not providing immunity. The best performing genotypes had a combination of potyvirus resistance QTL on chromosomes 3, 6 and 10. These three QTL were previously shown to be important in providing protection against SCMV (Zambrano et al., 2014), MDMV (Jones et al., 2007) and WSMV (Stewart et al., 2012). The potential role of two QTL interactions cannot be disregarded, as combinations of QTL 3 + 6 and 3 + 10 were also significantly better than controls.

Resistance to potyvirus is clustered in the maize genome (Redinbaugh & Pratt, 2009). Loci on the short arm of chromosome 6 and near the centromere of chromosome 3 have major effect on potyvirus resistance (Jones et al., 2007; Redinbaugh et al., 2004; Xia et al., 1999; Wang et al., 2003, Zhang et al., 2003; Zambrano et al., 2014).

The locus on chromosome 3 near the centromere at bin 3.04/3.05 in combination with other QTL confers resistance to many viruses including WSMV, SCMV, MMV and MCDV (Redinbaugh & Zambrano, 2014). The locus overlaps the position of translation factor eIF4e (Zambrano et al., 2014), involved in conferring virus resistance by producing proteins, which fail to interact with the virus (Gomez et al., 2009). Current studies on MLN resistance found other candidate genes for resistance to MLN on the same region (Gowda et al., 2015; 2018). Other candidate genes include those with a function predicted to restrict virus movement within the plant as demonstrated in arabidopsis by Chrisholm et al (2000). A locus on chromosome 3.05 is known to be responsible in plant defense against pathogens encodes nucleotide-binding site leucine-rich repeat (NBS-LRR) protein (Xiao et al., 2007).

Recently, the locus on chromosome 3 was identified among for QTL responsible for MCMV resistance in the Oh1VI RIL population others being the loci on chromosome 1, 2, and 10 (Jones et al., 2018). The study also denoted that the locus on chromosome 3 was near marker S3_37246834 which had the LOD score of 4.3 explaining 16% of the phenotypic variation and the locus on chromosome 10, which was centred at marker S10_134058628, had the LOD score of 9.0 explaining 11% of the phenotypic variation. These loci overlap the same region responsible for resistance to Potyvirus and other multiple virus families as explained by Zambrano et al. (2014). The identified locus on chromosome 2 was unique to the Oh1VI population centered on marker S2 _ 163825081 with a LOD score of 10 explaining 18% of phenotypic variance (Jones et al., 2018). Other studies have also mapped the region on chromosome 3 and 6 as potential candidate for marker assisted MLN resistance breeding (Gowda et al., 2018). Other recent studies have also suggested the need to focus improve resistance to both viruses causing MLN than focusing on the disease itself (Karanja et al., 2018).

Generally, results indicate the role of potyvirus resistance in MLN control. Although none of the genotype were immune to MLN there is differences in response of genotypes and QTL to MLN infection. Genotypes with all three potyvirus resistance QTL on chromosome 3, 6 and 10 had more resistance to MLN than genotypes with one of the above QTL. This lead to a conclusion that, there is a role played by potyvirus resistance in MLN control especially in reducing MLN effects. More studies are needed to know the exact role played by potyvirus resistance and how much MLN effects are reduced with the presence of potivirus resistance QTL. This will provide the basis for introgressing potyvirus resistance in East African maize germplasm and pave way for a holistic approach of controlling MLN in Sub Saharan Africa.

In carrying out future studies, especially in field conditions in East Africa, materials used should be adapted to tropical environment. The RIL populations used for the study were derived from Oh1VI and Oh28 which originate from temperate environment hence did not perform well in tropical environment. Agronomically, genotypes performed poorly compared to tropical controls in parameter measured such as plant height, ear height, yield and days to anthesis and silking. The gap between anthesis and silking was also big indicating materials were under physiological stress which could hinder reproduction and the plants were attacked by a lot of endemic disease such as maize streak virus and a variety of insects and vectors. This could affect the results and quality of the study especially when disease scoring is done until plants have reached maturity.

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