








## Article

# Agronomic Performance and Resistance to Maize Lethal Necrosis in Maize Hybrids Derived from Doubled Haploid Lines

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**Abstract:** Maize (*Zea mays* L.) is one of the most widely cultivated grain crops globally. In sub-Saharan Africa (SSA), it plays an important role in ensuring both food and income security for smallholder farmers. This study was conducted to (i) assess the performances of testcross hybrids constituted from maize lethal necrosis (MLN) tolerant doubled haploid (DH) lines under various management conditions; (ii) estimate the combining ability effects and determine the nature of gene action in the DH lines; and (iii) identify DH lines and testcross hybrids for resistance to MLN, high grain yield, and other important traits. Eleven DH lines were crossed with 11 single-cross testers using the line-by-tester mating design, and 115 successful testcross hybrids were generated. These hybrids, along with five commercial check hybrids, were evaluated across four optimum management conditions, two MLN artificial inoculations, and one managed drought environment in Kenya. Under each management condition, the effects of genotypes, environments, and genotype-by-environment interactions were significant for grain yield (GY) and most other traits. Hybrids T1/L3, T10/L3, and T11/L3 exhibited higher grain yields under at least two management conditions. A combining ability analysis revealed that additive gene effects were more important than non-additive effects for GY and most other traits, except for leaf senescence (SEN) and MLN disease severity score. DH line L3 exhibited a desirable general combining ability (GCA) effect for GY, while L5 was the best general combiner for anthesis date (AD) and plant height (PH) across all management conditions. DH lines L2, L6, and L7 showed negative GCA effects for MLN disease severity. Single-cross testers T11 and T10 were good general combiners for GY under all management conditions. Hybrids T2/L11, T9/L10, and T2/L10 demonstrated high specific combining ability (SCA) effects for GY under all conditions. This study identified DH lines and testers with favorable GCA effects for grain yield, MLN resistance, and other agronomic traits that can be used in breeding programs to develop high-yielding and MLN-resistant maize varieties. Better-performing testcross hybrids identified in the current study could be verified through on-farm testing and released for commercial production to replace MLN-susceptible, low-yield hybrids grown in the target ecologies.

**Keywords:** artificial inoculation; combining ability; doubled haploid; maize lethal necrosis; managed drought stress; *Zea mays*



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## 1. Introduction

Maize is among the most widely cultivated and economically important grain crops globally. It is an important source of income and food security for smallholder farmers in SSA. Over 4.5 billion people are reported to rely on maize for about 30% of their daily calorie intake in developing countries [1]. However, various stress factors, such as diseases, insect pests, weeds, drought, heat stress, and low soil fertility, significantly affect maize production and productivity [2–5], leading to reduced yields. In eastern Africa, the spread of maize lethal necrosis (MLN), which is a severe viral disease, has had a substantial impact on maize production and productivity [6]. This disease is caused by the co-infection of maize chlorotic mottle virus (MCMV) and sugarcane mosaic virus (SCMV) [6,7]. MLN can cause up to a 100% yield loss in maize production when conditions are favorable for the disease [5,7–9]. Moreover, the transmission methods of MLN can exacerbate the impacts of the disease. In addition to infected seeds and soils, thrips and beetles serve as insect vectors for MCMV, while aphids transmit SCMV [7,10–12].

Since the emergence of MLN disease in East Africa, CIMMYT, in collaboration with the Kenya Agricultural and Livestock Research Organization (KALRO), has established a quarantine site for MLN, screened over 100,000 lines from different sources, and identified a few promising MLN-resistant donor genotypes. Consequently, CIMMYT has been developing large numbers of MLN-resistant lines and hybrids each year. This process has been accelerated by integrating DH technology and marker-assisted introgression of MLN-resistant QTLs/genes into elite adapted lines [5,9]. A large number of DH lines were developed using elite lines of the CIMMYT regional breeding hubs in Africa and MLN donors, which were later evaluated through the artificial infestation of MLN viruses to identify several promising MLN-resistant lines [5].

Developing multiple stress-resilient maize genotypes is the ultimate objective of breeding programs in SSA. The preferred genotypes are those that exhibit tolerance to abiotic stresses such as drought and low soil nitrogen, as well as resistance to biotic stresses like MLN and other foliar diseases. However, different genotypes respond differently to various environmental conditions due to genotype-by-environment interactions [13,14]. Evaluating maize hybrids under MLN and managed drought conditions is crucial for identifying those resistant to MLN, as well as lines that possess favorable haplotypes for optimal performance under water stress conditions [2,15–17].

The line-by-tester design is commonly used by maize breeders to assess the GCA and specific combining ability (SCA) effects of newly developed parental inbred lines and hybrid combinations for traits of interest. It also helps to determine the significance of additive and non-additive gene effects that influence the expression of target traits [18–20]. Therefore, it is crucial to assess the breeding potential of newly developed MLN-resistant DH lines and determine the mode of gene action controlling the traits of interest. It is also equally important to evaluate the agronomic performances, MLN resistance, and trait heritability of the testcross hybrids derived from these DH lines. The objectives of this study were to (i) assess the performances of testcross hybrids constituted from maize lethal necrosis (MLN) tolerant doubled haploid (DH) lines under various management conditions; (ii) estimate the combining ability and determine the nature of gene action in the DH lines; and (iii) identify DH lines and testcross hybrids for resistance to MLN, high grain yield, and other important traits.

## 2. Materials and Methods

### 2.1. Parental Selection and Hybrid Formation

Eleven DH maize lines and 11 single-cross testers used in this study were sourced from CIMMYT-Kenya (Table 1). The DH lines were selected based on their per se phenotypic performances in the breeding nurseries and their resistance to MLN under artificial disease pressure. The lines and testers were mated following the line-by-tester mating design, resulting in 121 testcross hybrids. The DH lines served as the male parents, while the single-cross testers were used as the female parents. Due to inadequate seed quantities,

6 testcross hybrids were not included in the evaluation, leaving 115 successful testcrosses to be evaluated, along with five check hybrids (PH30G19, WH505, H516, DK8031, and DK777). DK777 was considered an MLN-tolerant check, while the other four hybrids were included as commercial checks for grain yield and agronomic traits, but the hybrids are susceptible to MLN.

**Table 1.** Description of the doubled haploid (DH) lines and testers used for this study.

Code	Name	Description
L1	CKDHL64076	Line
L2	CKDHL10310	Line
L3	CKDHL64665	Line
L4	CKDHL64672	Line
L5	CKDHL63598	Line
L6	CKDHL63908	Line
L7	CKDHL63943	Line
L8	CKDHL64302	Line
L9	CKDHL42833	Line
L10	CKSBL10060	Line
L11	CKDHL63627	Line
T1	CKLTI0138/CKLMARSI0022	Tester
T2	CKLTI0227/CKLMARSI0022	Tester
T3	CKDHL10918/CKLMARSI0022	Tester
T4	CKLTI0138/CML550	Tester
T5	CKDHL10918/CML494	Tester
T6	CKLTI0139/CKDHL10918	Tester
T7	CKLTI0227/CKDHL10918	Tester
T8	CKLMARSI0037/CML543	Tester
T9	CML543/CML494	Tester
T10	CML322/CML543	Tester
T11	CKDHL0500/CML543	Tester
CK1	PH30G19	Commercial MLN-susceptible check
CK2	WH505	Commercial MLN-susceptible check
CK3	H516	Commercial MLN-susceptible check
CK4	DK8031	Commercial MLN-susceptible check
CK5	DK777	MLN-tolerant check

## 2.2. Environments and Trial Management

Field evaluations of the 115 testcross hybrids and five checks were conducted in 2019 and 2020 across seven environments consisting of three different management conditions. These include four optimum management conditions (Kakamega, Kiboko, Kirinyaga, and Kaguru), one managed drought (Kiboko), and two seasons of MLN artificial inoculation at Naivasha, Kenya (Table 2). The experiment was conducted using a 10 × 12 alpha lattice design, with two replications in each environment. The entries were planted in a two-row plot that was 5 m long, with 0.75 m inter-row and 0.25 m intra-row spacings. Initially, two seeds were sown per hill, and then they were thinned to one plant per hill after two weeks of seedling emergence to attain the final plant population of 53,333 plants ha<sup>-1</sup>. Each experimental site received the recommended agronomic management and fertilizer rates (Table 2). One-half of the recommended nitrogen fertilizer was applied at planting, while the remaining half was applied six weeks after plant emergence. Hand-weeding was used to keep the fields weed-free throughout the cropping season.

**Table 2.** Description of experimental sites used to evaluate the testcross hybrids.

Location	Management	Year	Altitude m.a.s.l.	Latitude	Longitude	Fertilization (kg ha <sup>-1</sup> )	Grain Yield (t/ha)	
							Mean + SE	H <sup>2</sup>
Kakamega	Optimum	2019	1580	0°16' N	34°46' E	38 P, 93 N	9.02 + 1.26	0.82
Kiboko	Optimum	2019	1020	2°15' S	37°75' E	60 P, 87 N	7.67 + 0.86	0.77
Kiboko	Managed drought	2019	1020	2°15' S	37°75' E	60 P, 87 N	4.86 + 0.74	0.69
Kirinyaga	Optimum	2019	1159	0°34' S	37°19' E	50 P, 138 N	7.17 + 1.47	0.62
Kaguru	Optimum	2019	1460	0°02' N	37°39' E	50 P, 138 N	6.54 + 0.98	0.61
Naivasha	MLN-AI	2019	1896	0°43' N	36°26' E	60 P, 87 N	3.19 + 0.85	0.77
Naivasha	MLN-AI	2020	1896	0°43' N	36°26' E	60 P, 87 N	2.63 + 0.65	0.76

MLN-AI, maize lethal necrosis artificial inoculation; SE, standard error; H<sup>2</sup>, broad-sense heritability.

Trials at Kakamega, Kirinyaga, and Kaguru were conducted under rain-fed conditions, while the optimum trial at Kiboko and MLN trials at Naivasha were grown with irrigation. The trials evaluated under optimum management conditions received supplementary irrigation to avoid moisture stress during and after flowering until three weeks before harvesting. For the trial planted under managed drought stress condition at Kiboko, irrigation was discontinued two weeks prior to the estimated flowering date, and then the trial did not receive irrigation up until harvest.

### 2.3. Inoculum Production and Inoculation

MLN screening was conducted under artificial inoculation conditions at the KALRO-CIMMYT MLN screening facility in Naivasha. MCMV and SCMV were mass-produced in separate greenhouses following the MLN inoculation preparation and inoculation methods [9,10,21]. Briefly, MCMV and SCMV were extracted separately and then combined in a 1-to-4 ratio of MCMV–SCMV to create the MLN-causing inoculum. To ensure uniform plant inoculation, the first inoculation was carried out at the fourth week (V5 stage) after planting, and the second inoculation was carried out one week after the first. The MLN disease severity data used in this study were recorded at the silking stage of the plant.

### 2.4. Trait Assessment

Data were captured on grain yield, agronomic traits, and reaction to MLN disease at appropriate stages of the crop. The harvested ears from each plot were weighed, and the grain yield was determined using the field weight and grain moisture content adjusted to 12.5% at an 80% shelling percentage. Grain yield (GY) was then expressed in tons per hectare. The anthesis date (AD) and silking date (SD) were recorded as the number of days from planting to the date of 50% anthesis or pollen shed and 50% silking, respectively. The anthesis-silking interval (ASI) was recorded as the difference between the SD and AD. Following the milk stage, plant height (PH) and ear height (EH) were measured in centimeters (cm) from the ground level to the base of the tassel and to the node holding the uppermost ear, respectively. Ears harvested per plot were divided by the total number of plants in the plot at the time of harvesting to obtain the number of ears per plant (EPP). Husk cover (HC) was recorded as the proportion of plants with poor husk cover to the total number of plants in the plot and expressed as a percentage. Leaf senescence (SEN) was recorded under managed drought conditions at the grain filling stage on a scale of 1 to 10 based on the percentage of dead leaf area and then divided by 10 [2,17,22]. MLN disease severity (MLN-DS) was recorded from the trials planted under artificial MLN inoculation conditions using a 1 to 9 rating scale, where 1 stands for no visible MLN symptoms and 9 stands for complete plant death or necrosis [6,7,9,23].

### 2.5. Statistical Analysis

GenStat version 18 software was used to validate grain yield and other yield-related trait data for meeting the assumption of analysis of variance (ANOVA) [24]. ANOVA for all

traits was performed using META-R software [25]. A combined ANOVA was performed for optimum and MLN-infested environments separately, while a single environment ANOVA was used for managed drought stress. The best unbiased linear estimate (BLUE) and best linear unbiased prediction (BLUP) were obtained for each genotype at each location and across locations by using genotypes as fixed effects, and environments, replications, and blocks within replications were considered random effects [25]. BLUP-based mean values were used to present and interpret the results of this study. Variance components were estimated in META-R using a mixed model. The ratio of genotypic variance to phenotypic variance was used to estimate heritability ( $H^2$ ).

A line-by-tester analysis across environments was conducted using R software-based analysis of genetic designs (AGD-R) version 5.0 for traits that showed significant differences among hybrids based on adjusted hybrid means from each environment analysis, excluding checks. The total variations among the testcross hybrids were partitioned into lines (L), testers (T), and line  $\times$  tester (L  $\times$  T) sources of variations. The main effects of lines and testers represent the GCA effects, while L  $\times$  T interactions represent the SCA effects. In the combined analysis, the significance of line, tester, and L  $\times$  T mean squares were tested against the mean squares of their respective interactions with the environment. The mean square attributed to L  $\times$  T was tested against the mean square for L  $\times$  T interaction with environment (E), whereas the mean square for L  $\times$  T  $\times$  E was tested using the pooled error mean square. The GCA effects of lines ( $GCA_{Line}$ ) and testers ( $GCA_{Tester}$ ) and SCA effects of crosses (SCA) and their respective standard errors were determined using AGD-R. The relative importance of the GCA and SCA effects, as well as the corresponding gene actions, were determined by calculating the proportion of the GCA sum of squares for lines and testers or the SCA sum of squares to the total cross sum of squares.

### 3. Results

#### 3.1. Analysis of Variance and Hybrid Performance

Under optimum management conditions, highly significant variations were observed among the genotypes and environments for all studied traits (Table 3). Genotype-by-environment interaction (GEI) was also significant for most traits, except for AD and EH. Under MLN-AI conditions, the effects of genotypes and environments were significant for GY, AD, PH, and MLN-DS, but the GEI effect was significant only for GY and AD. Under managed drought stress condition, genotype mean squares were significant for GY and leaf SEN.

Broad-sense heritability among all studied traits ranged from 0.70 to 0.96 under the optimum management conditions (Table 3). High heritability was observed for EH (0.96), PH (0.94), AD (0.93), HC (0.86), and GY (0.85), while ASI (0.76) and EPP (0.70) showed relatively moderate heritability. Under MLN-AI, MLN-DS (0.90) and AD (0.83) had high heritability, while GY (0.74) and PH (0.66) showed moderate heritability. Similarly, AD (0.89) and PH (0.81) exhibited high heritability, while ASI (0.79), GY (0.69), and SEN (0.60) had moderate broad-sense heritability under managed drought condition (Table 3).

For all tested entries, the GY under optimum conditions ranged from 4.7 to 11.1 t ha<sup>-1</sup> with a mean of 7.6 t ha<sup>-1</sup> (Table 3; Supplementary Table S1). Under MLN-AI conditions, the GY ranged from 0.9 to 4.9 t ha<sup>-1</sup>, with a mean of 2.9 t ha<sup>-1</sup>. Under managed drought conditions, the GY ranged from 3.3 to 6.3 t ha<sup>-1</sup>, with a mean of 4.8 t ha<sup>-1</sup>. On average, significant GY reductions of 36 and 62% were observed under managed drought and MLN-AI conditions, respectively, compared to optimum management. The mean, minimum, and maximum values for all studied traits under different management conditions are presented in Table 3.

**Table 3.** Mean squares and heritability estimate for MLN disease severity, grain yield, and other agronomic traits in testcross hybrids of maize evaluated across seven environments, including optimum management, maize lethal necrosis artificial inoculation (MLN-AI), and managed drought stress conditions.

Source of Variation	Optimum Management								MLN-AI					Managed Drought					
	DF	GY	AD	ASI	PH	EH	HC	EPP	DF	GY	AD	PH	MLN-DS	DF	GY	AD	ASI	PH	SEN
Environment (E)	3	128 **	8292.1 **	719.43 **	19,288.2 **	22,933.8 **	14,626.8 **	0.99 **	1	9.7 **	10,974.6 **	39,130.9 **	73.4 **	-	-	-	-	-	-
Rep (Environment)	4	1.6	10.82	2.39	327.35	326.5	35.03	0.01	2	4.0 **	1350.8 **	9436.5 **	1.1	1	2.3 **	2279	125.6	19,412.3 **	6.78 **
Genotype (G)	114	13.2 **	17.2 **	14.11 **	656.7 **	562.5 **	2094.45 **	0.07 **	114	3.0 **	39.7 **	458.7 *	1.8 **	114	1.5 **	25.3	3.7	523.2	1.07 **
GCA <sub>Line</sub>	10	86.5 **	35.4 **	16.61 **	1264.4 **	1550.5 **	1724.68 **	0.09 **	10	17.6 **	40.9	390.7	1.1	10	6.3 **	7.5	2.4	229.7	3.52 **
GCA <sub>Tester</sub>	10	49.6 **	116.7 **	96.97 **	4542.6 **	3614.7 **	19,718.12 **	0.58 **	10	5.4 **	267.9 **	2541.6	8.4 **	10	4.9 **	235.3 **	21.1 **	4174.2 **	2.31 **
SCA	94	1.5 *	4.6	5.03 **	178.7	132.7	258.94 **	0.02	94	1.1 **	15.6	243.1	1.1 **	94	0.61	4.9	2	166	0.68
G × E	342	1.9 **	4.7	4.43 **	230.3 *	169.6	293.54 **	0.02 *	114	1.0 **	20.9 **	349.2	0.8	-	-	-	-	-	-
GCA <sub>Line</sub> × E	30	4.5 **	6	3.77 **	282.1	204.3	256.43	0.02	10	3.1 **	21.3	214.3	1.9	-	-	-	-	-	-
GCA <sub>Tester</sub> × E	30	3.7 **	12.8 **	6.68 **	840.7	509.1	1196.62 **	0.06 **	10	1.8 **	96.3 **	1817.2 **	0.7	-	-	-	-	-	-
SCA × E	282	1.4	3.7	4.26 *	159.8	129.8	201.41	0.02	94	0.7	12.9	207.8	0.7	-	-	-	-	-	-
Residuals	304	1.3	5.2	3.71	192	167	142.89	0.02	148	0.6	33	387.1	1.4	76	0.51	28.6	3.4	486.9	0.45
%SS GCA		0.91	0.78	0.71	0.78	0.81	0.83	0.78		0.69	0.68	0.56	0.48		0.66	0.84	0.56	0.74	0.48
%SS SCA		0.09	0.22	0.29	0.22	0.19	0.18	0.22		0.31	0.32	0.44	0.52		0.34	0.16	0.44	0.26	0.52
Mean		7.6	68.52	-0.09	259.00	131.96	22.09	1.05		2.91	87.18	166.15	3.38		4.86	70.61	-0.18	224.49	3.40
Minimum		4.69	63.67	-1.54	227.51	109.06	2.59	0.95		0.96	81.03	143.92	2.30		3.27	66.99	-1.95	194.82	2.46
Maximum		11.12	74.33	2.00	287.76	160.21	71.85	1.25		4.95	95.66	182.72	6.29		6.32	75.68	3.08	243.37	4.56
LSD <sub>0.05</sub>		1.38	1.67	1.16	9.93	7.89	19.03	0.13		1.26	4.09	17.48	0.68		1.27	1.89	1.45	14.00	1.13
CV (%)		15.1	1.89	21.40	2.95	4.98	54.68	12.13		26.1	3.42	6.45	11.68		15.3	1.33	10.24	3.15	20.59
Heritability (H <sup>2</sup> )		0.85	0.93	0.76	0.94	0.96	0.86	0.70		0.74	0.83	0.66	0.90		0.69	0.89	0.79	0.81	0.60

DF, degree of freedom; GY, grain yield (t ha<sup>-1</sup>); AD, anthesis date (day); ASI, anthesis-silking interval (day); PH, plant height (cm); EH, ear height (cm); HC, husk cover (%); EPP, number of ears per plant; MLN-DS, maize lethal necrosis disease severity (1 to 9 scale); SEN, leaf senescence (%); CV, coefficient of variation (%); LSD, least significant difference; \*, significant at  $p \leq 0.05$ ; \*\*, significant at  $p \leq 0.01$ .

Among the 25 top-yielding hybrids, the GY ranged from 7.8 to 11.1 t ha<sup>-1</sup> under optimum conditions, 4.7 to 6.3 t ha<sup>-1</sup> under managed drought conditions, and 2.3 to 4.2 t ha<sup>-1</sup> under MLN-AI conditions, with respective means of 8.7, 5.4, and 3.5 t ha<sup>-1</sup> (Table 4). These hybrids had 16, 39, and 147% GY advantage over the mean of checks under optimum, managed drought, and MLN-AI conditions, respectively. Under optimum conditions, the highest yielding hybrids were T11/L3 (11.1 t ha<sup>-1</sup>), T1/L3 (10.1 t ha<sup>-1</sup>), and T10/L3 (10.0 t ha<sup>-1</sup>). These hybrids had 27%, 15%, and 14% GY advantages over the best commercial check, PH30G19 under the same management conditions. Under managed drought conditions, hybrids T8/L3 (6.3 t ha<sup>-1</sup>), T2/L3 (6.2 t ha<sup>-1</sup>), T10/L3 (6.1 t ha<sup>-1</sup>), and T11/L3 (6.0 t ha<sup>-1</sup>) showed significantly higher GYs than the other hybrids. The hybrids showed 37, 35, 33, and 30% GY advantages over the best commercial check under managed drought condition, WH505, with a mean GY of 4.6 t ha<sup>-1</sup>. Under MLN-AI conditions, hybrids T1/L3 (4.2 t ha<sup>-1</sup>), T9/L7 (4.0 t ha<sup>-1</sup>), T7/L3 (4.0 t ha<sup>-1</sup>), and T4/L4 (3.9 t ha<sup>-1</sup>) had higher GYs than the MLN-tolerant commercial check, DK777 (2.4 t ha<sup>-1</sup>). Hybrids T11/L3 and T10/L3 showed higher GYs under both optimum and managed drought conditions, while T1/L3 had a higher GY under optimum management and MLN disease infestation conditions.

**Table 4.** Mean performance of the top-yielding 25 maize hybrids and five commercial checks evaluated under optimum management, managed drought stress, and maize lethal necrosis artificial inoculation (MLN-AI) conditions.

Hybrid Code	Tester/Line	Grain Yield Under					Agronomic Traits and MLN Disease Score					
		Optimum	Managed Drought	MLN-AI	AD	ASI	EPP	HC	PH	EH	SEN	MLN-DS
H23	T1/L3	10.1	5.4	4.2	70.1	-0.8	1.22	8.6	287.8	152.9	3.1	3.7
H24	T2/L3	8.7	6.2	3.6	69.4	-0.5	1.10	12.1	274.5	146.4	3.3	3.9
H26	T4/L3	9.2	5.4	3.5	70.0	-0.8	1.20	11.0	281	148.7	3.7	3.8
H27	T5/L3	8.1	4.9	3.7	71.2	-1.3	1.11	20.4	274.2	146.5	3.8	3.3
H28	T6/L3	8.1	5.4	3.5	71.6	0.4	1.08	15.3	278.9	145.9	3.5	3.5
H29	T7/L3	8.2	4.7	4.0	70.8	0.0	1.04	6.6	275.7	146	3.4	3.7
H30	T8/L3	9.1	6.3	3.4	70.5	0.8	1.07	11.9	272.8	144.6	3.1	4.2
H31	T9/L3	9.6	5.2	3.8	73.2	0.4	1.08	6.6	279.7	151.9	3.7	4.2
H32	T10/L3	10.0	6.1	2.8	71.8	-0.4	1.05	7.7	276.1	150.4	3.6	4.7
H33	T11/L3	11.1	6.0	2.3	72.3	-0.5	1.09	4.1	275.2	156.7	3.4	4.9
H34	T1/L4	8.5	5.5	3.0	68.7	-0.8	1.22	5.0	270.1	137.5	3.3	4.4
H35	T2/L4	8.4	5.4	3.7	67.7	-0.4	1.23	6.7	271.4	136.5	3.4	3.8
H37	T4/L4	8.3	5.3	3.9	68.3	-0.7	1.25	7.9	266	137.1	4.0	3.9
H42	T9/L4	9.3	5.9	3.8	70.1	-0.8	1.13	7.0	264.9	135.3	3.0	4.0
H44	T11/L4	9.6	5.6	3.6	69.7	-1.2	1.09	4.9	270.0	146.0	3.0	3.7
H59	T9/L6	8.0	5.3	3.8	68.9	-0.8	1.05	35.9	262.4	137.3	3.2	3.6
H70	T9/L7	7.8	5.5	4.0	67.5	0.0	1.02	25.3	263.0	135.9	3.5	3.6
H72	T11/L7	8.2	5.3	3.5	67.4	-0.8	1.01	17.2	254.7	137.4	2.8	3.7
H74	T2/L8	8.3	4.9	3.4	64.5	0.4	1.0	43.2	259.6	130.9	2.8	3.9
H80	T9/L8	8.4	5.2	3.7	67.7	0.3	1.02	42.5	267.9	143.7	3.6	3.6
H84	T2/L9	8.7	4.9	3.1	70.5	-1.3	1.24	3.5	282.2	152.3	3.1	4.8
H105	T1/L11	7.8	5.2	3.8	68.2	-0.9	1.10	64.6	264.2	127.8	3.0	3.8
H112	T8/L11	8.1	5.4	3.5	67.2	-0.5	1.00	46.5	260.1	128.1	2.9	4.0
H114	T10/L11	8.1	5.5	3.3	68.3	-1.6	1.02	61.1	257.5	130.1	3.4	3.7
H115	T11/L11	8.3	5.7	3.3	69.5	-1.9	1.06	22.5	261.9	137.5	2.8	4.5
CK1	PH30G19	8.8	3.8	1.2	68.2	3.1	1.0	6.5	265	117.7	3.1	6.1
CK2	WH505	8.7	4.6	1.3	73.1	-0.1	1.0	5.6	282.7	148.7	2.9	5.3
CK3	H516	6.1	3.3	1.3	71.6	3.0	1.0	10.2	283.8	154.2	4.2	5.9
CK4	DK8031	6.5	3.3	1.0	67.1	1.5	1.0	10.0	252.2	123.0	4.5	7.1
CK5	DK777	7.5	4.5	2.4	70.8	-1.2	1.0	11.1	255.8	123.9	3.2	5.2
Mean		8.7	5.4	3.5	69.4	-0.5	1.1	19.9	270.1	141.7	3.3	4.0
Minimum		7.8	4.7	2.3	64.5	-1.9	1.0	3.5	254.7	127.8	2.8	3.3
Maximum		11.1	6.3	4.2	73.2	0.8	1.3	64.6	287.8	156.7	4.0	4.9
LSD <sub>0.05</sub>		1.23	1.27	1.26	1.67	1.16	0.13	19.03	9.93	7.89	1.13	0.68

GY, grain yield (t ha<sup>-1</sup>); AD, anthesis date (day); ASI, anthesis-silking interval (day); EH, ear height (cm); PH, plant height (cm); HC, bad husk cover (%); EPP, number of ears per plant; SEN, leaf senescence (1 to 10 scale); MLN-DS, maize lethal necrosis disease severity (1 to 9 scale); CV, coefficient of variation; LSD, least significant difference at 0.05. Data for agronomic traits were recorded from optimum management trials, except for SEN and MLN. SEN data were recorded from managed drought trials, whereas MLN score was recorded from artificially inoculated trials at Naivasha.

The mean AD was 69 days, with a range of 64 days (T2/L8) to 73 days (T9/L3). Almost all the test hybrids showed lower ASI absolute values than the check hybrids, PH 30G19

(3.1 days) and H516 (3.0 days). Hybrids T9/L7 and T7/L3 had desirable ASIs of zero. The EPP ranged between 1.0 and 1.3, with a mean of 1.1. Hybrids T4/L4, T2/L9, and T2/L4 had high EPPs of 1.3, 1.2, and 1.2, respectively. The husk cover (HC) ranged from 3.5 to 64.6%, with a mean of 19.9%. The PH varied from 255 to 288 cm, with a mean of 270 cm, while the EH varied from 128 to 157 cm, with a mean of 142 cm. Check hybrids (DK8031 and PHB30G19) had the shortest plant (252 cm) and ear (117 cm) heights, respectively, compared to the top-yielding hybrids and all other commercial checks. Among the tested hybrids, T11/L7, T2/L8, and T10/L11 showed shorter PHs, while T1/L11, T8/L11, and T10/L11 had shorter EHs (Table 4).

Based on a 1 to 10 scoring scale, the SEN ranged from 2.8 to 4.0, with a mean of 3.3. Hybrids T11/L7, T2/L8, and T11/L11 had the lowest SEN (2.8) among all the hybrids and commercial checks. Under MLN artificial inoculation conditions, the MLN-DS score varied from 3.3 to 4.9, with a mean of 4.0 (Table 4). All the selected hybrids had lower disease scores than the commercial checks. Hybrids T5/L3 and T6/L3 had lower disease scores, whereas the commercial checks DK8031 and PH30G19 had higher MLN scores. The most tolerant commercial check, DK777, had an MLN score of 5.2.

### 3.2. Combining Ability Analysis

Under optimum management conditions, the line GCA and tester GCA mean squares were highly significant ( $p < 0.01$ ) for all studied traits, whereas the SCA mean squares were significant for GY, ASI, and HC (Table 3). Tester GCA  $\times$  E interaction mean squares were significant for most studied traits, except for PH and EH. On the other hand, line GCA  $\times$  E mean squares were significant only for GY and ASI, and significant SCA  $\times$  E interaction was observed for ASI. Under MLN inoculation conditions, the line and tester GCA effects were significant for GY. In addition, tester GCA mean squares were significant for AD and MLN-DS. SCA mean squares were significant for GY and MLN-DS. The mean square attributable to the line GCA  $\times$  E interaction effect was significant for GY, while tester GCA  $\times$  E interaction mean squares were significant for GY, AD, and PH. Under managed drought conditions, mean squares due to line GCA were significant for GY and leaf SEN, whereas tester GCA mean squares were significant for all studied traits (Table 3).

As indicated in Table 3, both GCA and SCA effects were involved in the inheritance of most studied traits. However, the GCA sum of squares, as a proportion of the hybrid sum of squares, was larger than the SCA sum of squares for all evaluated traits. The contributions of the GCA sum of squares to GY were 91% under optimum conditions, 69% under MLN-AI conditions, and 66% under drought conditions. The contributions of the GCA sum of squares were more than 50% under all management conditions for all studied traits, except for MLN disease severity and leaf SEN, which showed higher contributions of the SCA sum of squares of 52% (Table 3).

### 3.3. Estimates of GCA Effects

The estimates of GCA effects revealed that the contributions of the lines and testers to the testcross performances were highly variable for most traits across the management conditions (Tables 5 and 6). Under optimum management conditions, DH lines L3 and L9 had significant positive GCA effects for GY, while L5 had highly significant negative GCA effects. DH lines L1, L5, and L7 had desirable GCA effects for early anthesis. DH lines L6 and L7 showed significant negative GCA effects for ASI. L4 and L9 had highly desirable GCAs for EPP. L1 and L5 revealed desirable GCA effects for shorter plant stature. For HC, a significant negative GCA effect was observed for L11, whereas L3 and L9 had significant positive GCAs for HC under optimal management conditions (Table 5).

**Table 5.** Estimates of general combining ability (GCA) effects in 11 doubled haploid maize lines for gain yield, agronomic traits, and MLN disease severity under optimum management, maize lethal necrosis artificial inoculation (MLN-AI), and managed drought stress conditions.

Line	Optimum Management							MLN-AI			Managed Drought Stress					
	GY	AD	ASI	PH	EH	EPP	HC	GY	AD	PH	MLN-DS	GY	AD	ASI	PH	SEN
L1	-0.3	-0.90 *	-0.05	-4.87 *	-3.04	-0.05	-1.58	0.55 *	0.23	-1.63	0.18	0.53 *	-2.67 **	-0.76 **	-0.03	0.09
L2	-0.56	0.02	0.75 **	0.31	-4.54	-0.04	-0.62	-0.27	-0.72	1.6	-0.41 *	0.49 *	-3.26 **	0.16	8.52 *	-0.18
L3	1.19 **	1.75 **	0.00	12.63 **	10.47 **	0.04	5.73 *	0.65 *	2.87 **	8.14 *	-0.09	1.01 **	-1.78 *	-0.80 *	22.75 **	-0.03
L4	0.47	0.69	-0.13	4.05 *	1.64	0.06 **	-2.63	0.35	1.42 *	2.96	0.03	1.13 **	-1.78 *	-1.17 **	17.9 **	0.42 *
L5	-0.94 **	-1.41 **	-0.06	-15.1 **	-10.31 **	-0.03	3.48	-0.92 **	-2.38 **	-15.81 **	0.7 **	-0.04	-3.85 **	-0.81 *	-1.07	0.12
L6	-0.22	-0.44	-0.45 **	-2.52	-4.48	-0.04	6.36 **	0.37	-1.98 *	4.23	-1.19 **	0.26	-2.33 **	-0.6	8.36 *	0.52 **
L7	-0.18	-1.21 **	-0.38 *	-0.74	0.73	-0.01	3.04	0.55 *	-4.00 **	3.91	-0.59 *	-0.23	2.06 *	0.36	-12.87 **	0.18
L8	-0.01	-0.65	0.23	1.17	1.41	-0.02	-2.55	0.01	-2.18 *	7.29 *	-0.36	-0.88 **	3.54 **	1.26 **	-7.38	-0.64 **
L9	0.57 *	1.91 **	-0.05	7.58 *	8.05 **	0.08 **	-1.75	-0.96 **	5.92 **	-2.13	0.92 **	-0.85 **	4.47 **	1.16 **	-11.31 **	-0.11
L10	0.22	0.12	0.29 *	-1.85	3.26	0	0.35	-0.82 **	0.77	-11.85 **	1.04 **	-0.78 **	1.94 *	1.16 **	-7.66 *	0.03
L11	-0.23	0.12	-0.15	-0.67	-3.18	-0.01	-9.83 **	0.48 *	0.04	3.29	-0.23	-0.64 **	3.67 **	0.05	-17.20 **	-0.39 *
SE±	0.3	0.41	0.15	3.12	2.49	0.02	2.46	0.24	0.81	3.5	0.2	0.24	0.86	0.34	3.96	0.18

GY, grain yield (t ha<sup>-1</sup>); AD, anthesis date (day); ASI, anthesis-silking interval (day); PH, plant height (cm); EH, ear height (cm); EPP, number of ears per plant; SEN, leaf senescence (1 to 10 scale); HC, bad husk cover (%); MLN-DS, maize lethal necrosis disease severity (1 to 9 scale); SE, general combining ability standard error; \*, significant at  $p \leq 0.05$ ; and \*\*, significant at  $p \leq 0.01$ .

**Table 6.** Estimates of general combining ability effects in 11 single-cross testers for grain yield, agronomic traits, and MLN disease severity under optimum management, maize lethal necrosis artificial inoculation (MLN-AI), and managed drought stress conditions.

Tester	Optimum Management							MLN-AI			Managed Drought Stress					
	GY	AD	ASI	PH	EH	EPP	HC	GY	AD	PH	MLN-DS	GY	AD	ASI	PH	SEN
T1	0.39	-0.26	0.02	1.70	1.10	0.03	-8.83	0.37	-0.30	0.03	0.07	-0.12	-0.50	0.00	0.10	-0.25
T2	-0.03	-0.93 **	0.08	0.23	-1.75	0.01	-7.83	0.61	2.37 **	-0.06	0.27 *	0.16	0.51	-0.09	0.44	-0.32
T3	-0.74 *	-0.47	0.10	-6.57 **	-7.11 **	-0.02	-12.45 **	1.09 **	2.84 **	-0.05	0.17	-0.06	1.76 **	-0.34	-1.74	0.06
T4	-0.08	-0.49	-0.11	1.17	1.20	0.04	-15.1 **	0.05	-1.92 *	0.04	0.16	-0.14	-1.46 **	0.07	-1.65	-0.11
T5	-0.55	0.06	-0.16	-2.01	-2.05	0.00	3.29	-0.93 **	-1.66 *	0.00	-0.23 *	0.01	-0.23	-0.10	-2.06	0.46 *
T6	-0.44	0.08	-0.11	-1.30	-1.71	-0.01	11.91 *	-0.03	-2.13 *	0.04	-0.14	-0.5 **	-0.95 *	-0.05	-5.02	0.24
T7	-0.50	-0.20	0.06	-1.60	-1.61	-0.03	6.17	-0.81 *	-0.46	0.03	-0.23 *	0.00	0.44	-0.28	-0.12	-0.41 *
T8	0.43	-0.01	0.10	1.15	-0.18	0.00	17.17 **	-0.70 *	-0.34	0.02	-0.11	0.30	-0.03	0.04	2.23	0.05
T9	0.36	0.87 *	0.02	4.06 **	4.78 **	0.00	-19.19 **	-0.80 *	0.56	-1.36	0.02	-0.24 *	0.06	-0.31	2.89	0.01
T10	0.43	0.24	0.01	-0.22	0.58	-0.02	-11.6 *	0.60	0.47	-0.07	0.15	0.05	-0.16	0.61 **	0.68	0.12
T11	0.73 *	1.11 **	-0.02	3.40	6.74 **	0.00	36.47 **	0.60	2.48 **	0.00	0.12	0.24	0.94 *	0.17	4.26	0.15
SE±	0.27	0.3	0.1	1.83	1.73	0.16	4.65	0.31	0.77	0.04	0.14	0.19	0.34	0.18	2.23	0.17

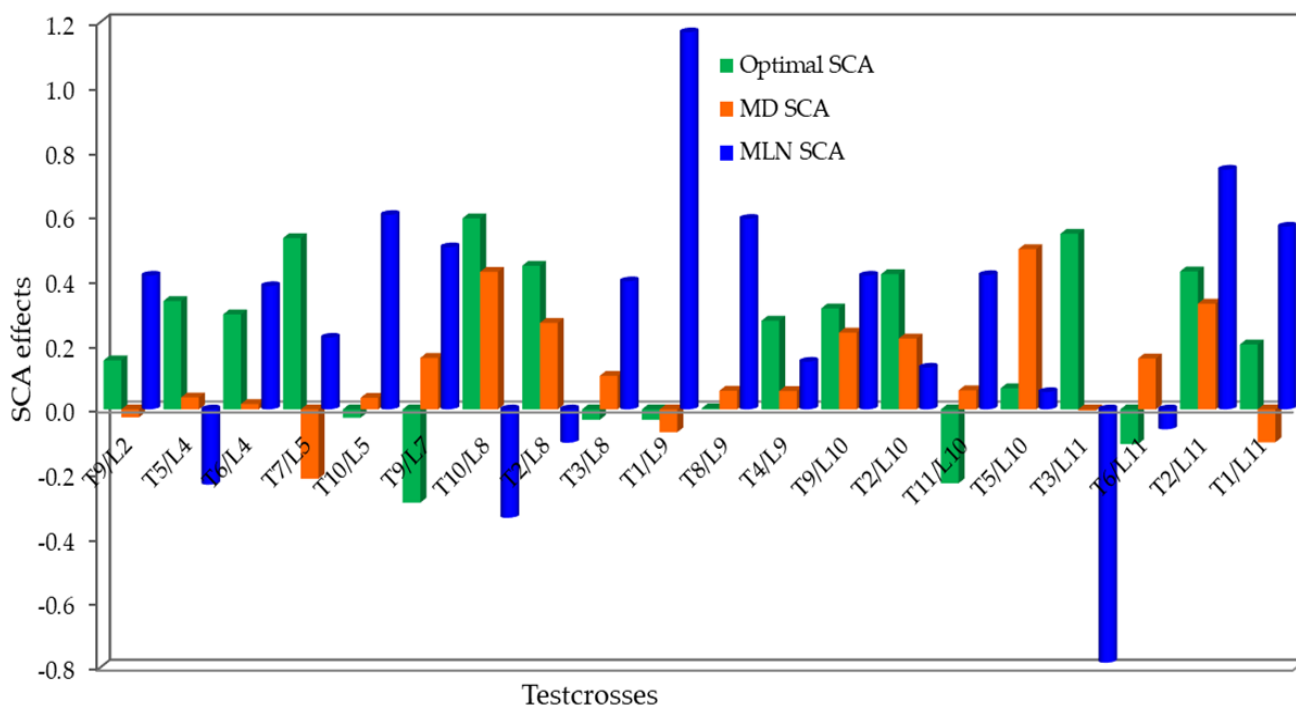
GY, grain yield (t ha<sup>-1</sup>); AD, anthesis date (day); ASI, anthesis-silking interval (day); PH, plant height (cm); EH, ear height (cm); EPP, number of ears per plant; SEN, leaf senescence (1 to 10 scale); HC, bad husk cover (%); MLN-DS, maize lethal necrosis disease severity (1 to 9 scale); SE, general combining ability standard error; \*, significant at  $p \leq 0.05$ ; and \*\*, significant at  $p \leq 0.01$ .

Under MLN-AI conditions, DH lines L1, L3, L7, and L11 had significant positive GCA effects for GY, whereas L5, L9, and L10 showed significant negative GCA effects (Table 5). For AD, L5, L6, L7, and L8 showed significant negative GCA effects. Lines L5 and L10 were good combiners for PH, as exhibited by significant negative GCA effects, while lines L3 and L8 had significant positive GCA effects. DH lines L2, L6, and L7 showed significant negative GCA effects for MLN-DS. Under managed drought condition, lines L1–L4 had significant positive GCA effects for GY, whereas lines L8–L11 showed undesirable GCA effects for GY (Table 5). Lines L1–L6 had highly significant negative GCA effects for ASI, while lines L1, L2, L5, and L6 had highly significant negative GCA effects. For PH, lines L7–L11 had significant negative GCA effects under managed drought condition. DH lines L8 and L11 showed significant negative GCA effects for leaf SEN.

Among the testers, T11 had a desirable GCA effect for GY, while T2 was a good combiner for early anthesis under optimum management conditions (Table 6). For plant and ear height, T3 had the most desirable GCA effects. DH lines T3, T4, and T9 showed highly significant negative GCA effects for HC. Under artificial MLN disease inoculation conditions, T3 had a highly significant positive GCA effect for GY, while testers T4–T6 showed negative and significant GCA effects for AD. T5, T7, and T9 showed significant negative GCA effects for MLN-DS (Table 6). None of the testers showed significant GCA effects for GY under managed drought condition. Testers T4 and T6 had significant negative GCA effects for AD, and T7 showed a significant negative GCA effect for leaf SEN.

### 3.4. Estimates of SCA Effects

The estimates of SCA effects for GY in selected cross combinations under various management conditions are presented in Figure 1. Testcross hybrids T2/L11, T9/L10, and T2/L10 showed specific combining ability effects for GY under all management conditions. Hybrids T10/L8 and T2/L8 were the best specific combiners under optimum and managed drought conditions, while T6/L4, T7/L5, and T1/L11 had significant positive SCA effects for GY under optimum and MLN-AI conditions. On the other hand, hybrids T9/L7, T5/L10, and T8/L9 were good specific combiners under managed drought and MLN-AI conditions.



**Figure 1.** Estimates of specific combining ability (SCA) effects for selected testcross hybrids for grain yield under optimum, managed drought stress (MD), and maize lethal necrosis (MLN) artificial inoculation conditions.

### 4. Discussion

This study evaluated the testcross performance and combining ability of DH lines under optimum, MLN-AI, and managed drought conditions. The significant variations observed among the genotypes for most studied traits (Table 3) indicated the possibility of making selections to identify preferred hybrids in target environments. Significant variations for GY and other agronomic traits under optimum and managed drought stress conditions were previously reported [26,27]. Similarly, the variations among environments suggest that each environment possesses unique characteristics that influence the performances of testcross hybrids. Consistent with this study, significant effects of the environment on GY and other agronomic traits were reported by other investigators [26,27]. As expected, considerable GY reductions were observed under MLN-AI and managed drought conditions compared to the optimum management conditions. In agreement with the current results, several researchers reported the effects of drought stress and MLN disease on maize productivity [4,7,21]. Most measured traits exhibited high broad-sense heritability ( $\geq 0.60$ ) under different management conditions, indicating that these traits are highly heritable and suggesting that selection for improvement would be effective. These heritability estimates could be categorized as high heritability [28] or moderately high to very high [29,30]. The high heritability estimates observed in this study suggested that genetic variations among the studied genotypes were greater than environmental

variations. MLN disease-tolerant maize genotypes with moderate to high heritability were recommended as potential donors [10].

Testcross hybrids that showed desirable GY, agronomic performance, and MLN disease resistance under diverse management conditions could be recommended for release and commercialization, including in MLN-prone areas. Hybrids derived from DH lines show noticeably higher GY advantages than the mean of the test hybrids, as well as the mean of commercial checks, under both optimum management and managed drought stress conditions [31–33]. Under drought and optimum management conditions, a substantial difference in hybrid yield was also reported by [32,33]. In addition to GY, substantial variations for AD, EH, and PH among newly developed maize hybrids were previously reported under drought stress and non-stress conditions [34–36]. Significant variations observed among the hybrids for MLN disease severity under high MLN disease pressure showed the potential for identifying hybrids with resistance or tolerance reaction to MLN. Hybrids T1/L3, T9/L7, T7/L3, and T4/L4, which showed higher GYs and more tolerance to MLN-DS than the MLN-tolerant hybrid check (DK777), could be considered for possible release in MLN disease-prone environments.

The significant differences observed among the testcross hybrids for SEN under managed drought stress condition suggest variation in the stay-green characteristics of the hybrids under moisture deficit conditions. This finding aligns with previous studies that reported variations among maize hybrids in their tolerance to drought stress [4,17,37–39]. Genotypes with stay-green characteristics under drought stress conditions have consistently been reported as indicators of drought tolerance [40]. A relative increase in GY and delayed SEN under drought stress conditions is attributed to higher nitrogen accumulation in the leaves and an extended photosynthesis process [41].

Although both additive and non-additive gene effects were important in the heritance of all the studied traits, additive gene effects had more contributions to the performance of hybrids. This indicates that hybrid performance could be adequately predicted based on the performance of lines and testers under optimum management condition, MLN disease pressure, and managed drought conditions. The importance of both additive and non-additive genetic effects in the inheritance of leaf SEN and MLN-DS suggests that improvement for these traits could be feasible through recurrent selection and systematic hybridization [10]. Additive gene action was found to be more important than non-additive gene action for MLN disease severity measured at both the early and late stages of the plant [12].

The significant GCA mean squares of lines and testers observed for GY and other traits under different management conditions suggested the presence of a high level of genetic diversity among the DH lines and single-cross testers used in this study. Like this finding, significant GCA effects among inbred lines evaluated for various traits in managed drought and optimum environments were previously reported [4,33,42,43]. This study identified several DH lines with desirable GCA effects for GY, MLN resistance, and other agronomic traits under each and across different management conditions, depicting that these DH lines could be useful sources of favorable alleles for these traits. DH line L3 had significant positive GCA effects under all management conditions, indicating that this line has favorable alleles for higher grain yield potential under diverse environmental conditions. DH lines L5 and L6 were the best general combiners for AD, whereas L5 and L10 had favorable alleles for shorter plant stature. L4 and L9 had desirable GCA effects for EPP and can be sources of favorable alleles for developing hybrids with high prolificacy. DH lines L8 and L11 can serve as sources of desirable alleles for stay-green characteristics under drought stress conditions, as depicted by negative and significant GCA effects for leaf SEN. L2, L6, and L7 had desirable GCA effects for MLN-DS under artificial MLN pressure conditions, thereby justifying the values of these lines in developing MLN-resistant maize varieties. Similarly, previous studies identified maize lines with desirable GCA effects for various agronomic attributes under different management conditions [33,44].

Among testers, T11 and T10 were good general combiners for GY under all management conditions. This study also identified testers with desirable GCA effects and, hence, favorable additive effects for AD (T4) and OH and EH (T3). Testers T5, T7, and T9 showed desirable GCA effects for MLN resistance. T7 has highly desirable GCA effects for drought tolerance, as shown by its low GCA effect for leaf SEN under managed drought conditions. In general, desirable GCA effects observed in the inbred lines and testers evaluated for most traits witness the breeding potential of the parents, which is largely due to additive genetic effects. This allows breeders to predict progeny performance based on the performance of the parents. Previous studies also reported inbred lines and testers with desirable GCA effects for GY, agronomic traits, and MLN-DS [12,45].

In hybrid breeding, it is important to identify a specific combination of inbred lines that would produce the best-performing hybrids in stressful and non-stressful environments. Variable SCA effects were observed among the cross combinations for grain yield under different management conditions, indicating the deviation of cross performances from what could be expected based on the GCA effects parents. Testcross hybrids T2/L11, T9/L10, and T2/L10 were the best specific combiners for GY under all management conditions.

## 5. Conclusions

The results of the current study showed high levels of genetic variation for GY, agronomic traits, and MLN resistance among the testcross hybrids derived from DH lines, indicating the possibility of genetic improvement for these traits through selection. Hybrids T1/L3, T10/L3, and T11/L3, with high GY potential, and hybrids T5/L3 and T6/L3, which were resistant to MLN disease, can be recommended for further testing and commercialization. Improvements in GY and MLN resistance observed in the current study indicated the genetic progress made by CIMMYT and its partners in developing MLN-resistant and high-yielding germplasm. DH lines and testers identified in this study with highly desirable GCA effects can be used as sources of favorable alleles for developing preferred varieties for the target environments.

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/agronomy14102443/s1>: Supplementary Table S1. Mean grain yield, maize lethal necrosis (MLN) severity, and other traits of 115 testcross hybrids and five commercial checks evaluated under optimum, MLN artificial inoculation, and managed drought stress environments in Kenya.

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**Data Availability Statement:** All relevant data are included in this article and its supplementary files. Raw data that support the findings of this study are available from the senior author upon request.

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## Abbreviations

AARC	Ambo Agricultural Research Center
ASI	anthesis-silking interval
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo (International Maize and Wheat Improvement Center)
DA	days to anthesis
DS	days to silking
EH	ear height
EIAR	Ethiopia Institute of Agricultural Research
EPP	ears per plant
GCA	general combining ability
GY	grain yield
H <sup>2</sup>	broad-sense heritability
HC	bad husk cover
KALRO	Kenya Agricultural and Livestock Research Organization
L	line
L × T	line-by-tester
MCMV	maize chlorotic mottle virus
MLN	maize lethal necrosis
MLN-AI	maize lethal necrosis artificial inoculation
MLN-DS	maize lethal necrosis disease severity
PH	plant height
SCA	specific combining ability
SCMV	sugarcane mosaic virus
SEN	leaf senescence
SSA	sub-Saharan Africa
T	tester
UG	University of Ghana
WACCI	West Africa Center for Crop Improvement

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