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RESEARCH ARTICLE

GENOTYPE BY ENVIRONMENT INTERACTION (GEI) OF MAIZE VARIETIES RESISTANT TO MAIZE LETHAL NECROSIS FOR MID ALTITUDES OF RWANDA

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ABSTRACT

Maize is a leading crop in Rwandan agriculture, but its production is threatened by the outbreaks of Maize Lethal Necrosis (MLN) virus disease. The establishment of MLN artificial inoculation facility in the region where Rwanda is located has facilitated the developing MLN resistant maize genotypes. The objective of this study was to identify maize MLN resistant varieties that are high yielding and stable across environments, and to integrate them with other disease management strategies in Rwanda. Forty-one maize varieties were screened through MLN artificial inoculation facility in Naivasha, Kenya. Then, 24 of them were evaluated under natural inoculation in Karama research station, Rwanda. Twelve potential maize varieties were investigated for genotype by environment interaction in four sites at mid-altitudes in Rwanda, including Cyabayaga Karama and Bugarama, over two consecutive seasons. The study identified three maize MLN moderately resistant varieties; RHM1402, RHM1407, and RHM1409 that are high yielding and stable across environments. MLN was found to increase the incidence of ear rot, hence, raising the risks of infection with harmful mycotoxins such as aflatoxins. In order to manage the MLN disease in Rwanda, it has been advocated that the identified varieties be made available to farmers and integrated with other methods of control.

Keywords: AMMI, AUDPC, Maize Chlorotic Mottle Virus, Maize Lethal Necrosis, Sugar Cane Mosaic Virus, Variety × Environment Interaction

INTRODUCTION

Maize (*Zea mays* L.) has become a staple food crop in Rwanda. In the year 2020, around 549,000 tons of maize grains were produced in the country (NISR 2020). The crop commodity is contributing to the generation of revenues. Approximately 50,800 tons of maize products (mainly flour and grains) were exported in the fiscal year 2018-2019 and generated 14.6 million US dollars (NISR 2019).

In the past 15 years, maize farming in Rwanda has undergone enormous growth and radical change. The most significant factors behind this great progress in a short period of time include the advent of the Crop Intensification Program (CIP) in 2007 (Nahayo *et al.* 2017), changes in cropping systems, extension and intensive cultivation of maize in mid-altitudes, changes in policies (Bizoza and Byishimo 2013), and availability of markets at national and regional levels (FAO 2013).

The outbreak of MLN (Maize Lethal Necrosis) disease in 2013 (Adams *et al*. 2014) posed a serious threat to maize production and maize cropping achievement in Rwanda. The MLN is a viral disease caused by a combined and synergetic infection of MCMV (Maize

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Chlorotic Mottle Virus) and one of the three cereal viruses of the *Potyviridae* family comprising SCMV (Sugarcane Mosaic Virus), WSMV (Wheat Streak Mosaic Virus) or MDMV (Maize Dwarf Mosaic Virus) (Mahuku *et al.* 2015a; Wamaitha *et al.* 2018; Redinbaugh and Stewart 2018). In Eastern Africa, the combination of MCMV and SCMV has been commonly reported (Wangai *et al.* 2012; Adams *et al.* 2014; Mahuku *et al.* 2015b; Kiruwa *et al.* 2016) although there is evidence that the combination of MCMV with Johnsongrass mosaic virus or polerovirus causes MLN as well (Stewart *et al.* 2017; Massawe *et al.* 2018).

Diseased plants develop several symptoms that include chlorotic mottle on the leaves starting from the base of the young leaves in the whorl and extending upwards toward the leaf tip, mild to severe leaf mottling, dwarfing and premature ageing of the plants, necrosis of leaf margins that progress to the mid-rib resulting in drying of the whole leaf, and necrosis of young leaves in the whorl before expansion. Severely infected plants show dead heart symptoms and eventual plant death (Redinbaugh and Stewart 2018; Awata *et al.* 2019).

Other important symptoms include immature ear husks showing yellow streaks, ears appearing physiologically mature while kernels inside are still at the milk stage, and the rest of the plant is still green before finally drying and rotting. Also, plants affected form small cobs with few and no grain at all resulting in barren ears (Wangai *et al.* 2012). Losses caused by MLN on maize crops can go up to 100 % (Boddupalli *et al.* 2020).

MLN disease is transmitted through a complex of means that include vectors, mechanical transmission, seeds, and agronomic practices depending on type of the viruses causing the disease. MCMV transmission occurs through insect vectors, mechanical means and seeds (Zhang *et al.* 2011; Zeng *et al.* 2013; Regassa *et al.* 2021). Although thrips (*Frankliniella williamsi*) seem to be the major insect vector transmitting MCMV, other possible vectors include three species of maize rootworms (*Diabrotica undecimpunctata*, *D. lonicornis* and *D. virgifera)*, the maize flea beetle (*Chaetocnema pulicaria*), the flea beetle (*Systena frontalis*), and the cereal leaf beetle (*Oulema melanopa*) (Cabanas *et al.* 2013; Awata *et al.* 2019; Regassa *et al.* 2021). All these insect vectors transmit MCMV in a semi -persistent manner for up to six days (Cabanas *et al.* 2013; Mwando *et al.* 2018). The mechanical transmission of MCMV happens through agricultural tools and the transport of infected plant parts to non-infected areas (Redinbaugh and Stewart, 2018).

SCMV and MDMV are mainly transmitted by several aphid species that include the maize leaf aphid (*Rhopalosiphum maidis)*, the plum aphid (*Hysteroneura setariae),* the green bug (*Schizaphis graminum),* the cotton aphid *(Aphis gossypii)* and the green peach aphid *(Myzus persicae)* in a non-persistent manner (Awata *et al.* 2019; Regassa *et al.* 2021). Furthermore, the two viruses are importantly spread through seed (Mikel *et al.* 2008). WSMV is transmitted from infected to healthy plants mainly by the mite, *Aceria tosichella* (Lu *et al.* 2011) causing wheat leaf curl through seeds (Dwyer *et al.* 2007; Hadi *et al*. 2011).

The MLN disease infects exclusively maize crop. However, its components viruses infect several important cereal crops worldwide such as maize, wheat, sorghum, and sugarcane and have several other hosts including wild species. Maize and sorghum seem to be the only natural host of MCMV (Zhang *et al.* 2011; Awata *et al.* 2019; Regassa *et al.* 2021).

The control measures of MLN include rigorous disease management practices such as crop rotation and fallowing where farmers would stop growing maize for a certain period of the time, and plant a non-grass crops such as legumes. Other measures include avoidance of continuous maize cultivation, timely planting and weeding, applying correct plant spacing, adequate fertilizer application for maximum plant health (Marenya *et al.* 2018), and the avoidance of use of seed produced in both MCMV and MLN non-infected zones (Zeng *et al.* 2013). Moreover,

they comprise agronomic practices such as control of weeds and alternate hosts, avoidance of mechanical transmission through tools, control of insect-vectors, use of resistant varieties, adequate isolation from MLN infected fields, and avoidance of transport of parts of maize crop from infected areas (Mahuku *et al.* 2015a).

The control of the seed movement from a MLN endemic region to a non-endemic zone is also a valuable practice that limits the spread of MLN. The national plant protection organizations have a crucial responsibility in ensuring that there is no movement of commercial seed from MLN-endemic to non-endemic countries or regions by issuing MLN -free seed certificates (Marenya *et al.* 2018).

The MLN disease was first reported in the USA where it has heavily infected three states: Hawai, Texas, and Nebraska (Bockelman *et al.* 1982). Furthermore, it has been reported to be heavily present in China (Zhang *et al.* 2011; Xie *et al.* 2010) where it has become a quarantine pest (Zeng *et al.* 2013). The first report of MLN and MCMV in Eastern and Central Africa was made in 2011 in Kenya (Wangai *et al.* 2012; Kiruwa *et al.* 2016) and rapidly spread to Tanzania, Uganda and Rwanda, D.R. Congo, and Ethiopia (Lukanda *et al.* 2014; Adams *et al.* 2014; Mahuku *et al.* 2015b; Mahuku *et al.* 2015a). In Rwanda, the symptoms of MLN disease were observed for the first time in February 2013 in Busogo Sector, Musanze District, Northern Province in volcanic highlands (Adams *et al.* 2014). From this initial outbreak, it spread quickly in the whole country with the volcanic highlands being the most infected, causing heavy losses to maize cultivation (Asiimwe *et al.* 2019).

Right from the beginning of the outbreak of MLN in Eastern and Central Africa, the International Maize and Wheat Improvement Center (CIMMYT) in collaboration with Kenya Agriculture and Livestock Research Organization (KALRO) established provisional artificial inoculation facilities in Kenya at Narok [latitude 01°05'S, longitude 35°52'E, 1827 m above sea level (masl)], and permanent MLN screening facilities in Kenya, Naivasha (latitude 0°43'S, longitude 36°26'E, 1896 masl). With the availability of artificial inoculation facilities, CIMMYT in collaboration with regional National Agricultural Research Systems (NARs) launched an aggressive drive to develop MLN tolerant or resistant varieties (Gowda *et al.* 2015).

The availability of the MLN screening facilities allowed the development of several MLN-resistant inbred lines through conventional breeding methods including pedigree methodology, molecular assisted backcrossing, and forward breeding. The availability of these inbred lines permitted the generation of new maize hybrid varieties tolerant or resistant to disease. A number of screening sites at MLN hot spots with high natural disease pressure were established and used to evaluate pre-commercial MLN-tolerant or resistant hybrids identified at the MLN screening facilities at Naivasha in Kenya. Through this network, maize hybrid varieties with MLN tolerance and or resistance combined with desirable traits, including excellent husk cover, reduced ear rots, and tolerance to other stresses were identified and deployed to manage the disease. Some of these varieties such as Bazooka in Uganda, D.R. Congo and Burundi, and H6506 in Kenya are being commercialized (Beyene *et al.* 2011; Boddupalli *et al.* 2020; Awata *et al.* 2021).

The genotype by environment interaction (GEI) occurs when genotypes differ in the manner their trait values vary across environments (Saltz *et al.* 2018). The environments may comprise locations, years, levels of fertilization, different plant density, and many more. GEIs can be grouped into two broad categories: crossover and non-crossover interactions. A crossover interaction occurs when variety ranks change from one environment to another while non-crossover interaction occurs when rank orders of genotypes across environments remains unchanged, *i.e.* genotypes that are superior in one environment maintain their superiority in other environments. The GEI has been

expressed as P=G+E+GE where P was the phenotype, G, the sum of the genotypic contribution, E, environmental contribution and GE, the interaction between genotypes and environments (Kang 2002).

Several statistical models to analyze the GEI have been developed and utilized. They range from linear models such as the joint regression developed by Finlay and Wilkinson (1963) to linear- bilinear models such as Additive Main Effect and Multiplicative Interaction (AMMI) (Gauch 1992; Gauch 2013), and Genotypes + Genotypes × Environments Interaction (GGE) models (Yan *et al.* 2005). The linear-bilinear models have two components: an additive (linear) component (main effects, intercepts) and a multiplicative (bilinear) component (Crossa and Cornelius 2002; Yang 2014).

The AMMI and GGE models have been extensively used to analyze and interpret the GEI, and identify genotypes stable across environment on maize and other several cop commodities. The advantages of one model over the other are still under debate. However, the advantage of AMMI over GGE seems to be the incorporation of yield in the concept of stability whereas the advantage of GGE over AMMI appears to be its powerful graphical representation through biplots (Yan and Tinker 2005; Gauch 2006; Yan *et al.* 2007; Gauch *et al.* 2008; Gauch 2013).

The AMMI combines classical analysis of variance and principal components analysis in one single analysis with both additive and multiplicative parameters. The AMMI linear model is:

$$Y_{ger} = \mu + \alpha_g + \beta_e + \sum_{n=1}^{N} \lambda_n \gamma_{gn} \delta_{en} + \rho_{ge} + \varepsilon_{ger}$$

.

The additive part of the model coincides with the ordinary analysis of variance and it is made of the grand mean μ and the main effects α_g: genotype deviation from the grand mean, β_e: environment deviation from the grand mean. The multiplicative part of the model decomposes the interaction into Principal Component Axis (PCA) and residual ρ_{ge} if all PCAs are not included in the model. The multiplicative parameters are λ_n the singular value for the nth PCA, γ_{gn} the genotype eigenvector for axis *n,* and δ_{en} the environment eigenvector for axis *n*. The multiplicative parameters are obtained by Singular Value Decomposition (SVD) of interaction. A convenient scaling for the multiplicative parameters is $\lambda^{0.5}\gamma_g$ and $\lambda^{0.5}\delta_e$. However, these multiplicative terms have been called Interaction PCA scores or IPCA scores in order to make a distinction between classical principal analysis and AMMI (Gauch 1992).

The distinctive feature of the AMMI model is the use of biplots to present the results of the analysis in a fashion that provides visual clarity and demonstrates the inherent patterns uncovered. A biplot is a scatter plot with two kinds of points. The biplot for GEI has one type of points that represents genotypes and another type that represents the environments. Most biplots currently encountered are drawn in two dimensions and are based on AMM1 and AMMI 2. For those based on AMM1, the means of main effects (genotypes and environment) constitute the abscissa axis and the IPCA1 make the ordinate axis whereas, for those based on AMMI 2, the IPCA1 scores make the abscissa axis and the IPCA 2 scores form the ordinate axis (Gauch 2006; Gauch *et al.* 2008; Gauch 2013).

The usefulness of the AMMI model has been reflected in the use of the yield stability index to obtain genotypes that are high yielding and stable across environments. It is obtained by adding the ranks of AMMI stability values (ASVs) in ascending order and the ranks of yield in descending order (Farshadfar *et al.* 2011; Oliveira *et al*. 2013; Oyekunle *et al.* 2017; Katsenios *et al.* 2021). The ASVs are obtained using the formulae:

ASY=

$$\sqrt{\left[\frac{IPCA1_{SS}}{IPCA2_{SS}} \times (IPCA1_{Score})\right]^2 + (IPCA2_{score})^2}$$

where the ASV is the AMMI stability value, $IPCA1_{SS}$ is the sums squares of IPCA1 in the

AMMI analysis of variance, $IPCA2_{SS}$ is the sums squares of IPCA2 in the AMMI analysis of variance, $IPCA1_{Score}$ is the IPCA1 score and $IPCA2_{Score}$ is the IPCA2 score. The ASVs close to zero indicates high stability.

The objective of this study was to identify MLN tolerant or resistant varieties which are high yielding and stable across environments that could be integrated with other control options to minimize the impact of MLN disease in Rwanda.

MATERIALS AND METHODS

Screening under MLN artificial inoculation

Forty-one hybrid maize varieties were generated using parent inbred lines developed by Rwanda Agriculture and Animal Resources Development Board (RAB) and inbred lines introduced from CIMMYT at Cyabayaga research station (latitude 1°24'25''S,

Table 1: Characteristics of varieties generated using RAB and CIMMYT inbred lines

No	Names	Pedigrees	Origin of parent 1	Origin of parent 2	Origin of parent 3
1	RHM102	CML442/CML440/CML445	CIMMYT	CIMMYT	CIMMYT
2	RHM104^{+}	CML442/CML444/CML445	CIMMYT	CIMMYT	CIMMYT
3	RHT132^{++}	CML202/CML204//CML216	CIMMYT	CIMMYT	CIMMYT
4	RHT132P	CML 203/CML 204	CIMMYT	CIMMYT	-
5	RHT104P	CML 442/CML 444	CIMMYT	CIMMYT	-
6	RHT131^{++}	CML208/CML202//CML216	CIMMYT	CIMMYT	CIMMYT
7	RHMM119	RML0004/RML0012//CML445	RAB	RAB	CIMMYT
8	RHMM113^{++}	RML0004/RML0010//CML488	RAB	RAB	CIMMYT
9	RHM1405	CML536/CML489//CML440	RAB	RAB	CIMMYT
10	RHMM115^{+}	RML0004/RML0011//CML445	RAB	RAB	CIMMYT
11	RHM1409^{++}	CML539/CML444/CML488	CIMMYT	CIMMYT	CIMMYT
12	RHMM128^{+}	RML0005/RML0011//CML488	RAB	RAB	CIMMYT
13	RHMM121	RML0004/RML0012//CML488	RAB	RAB	CIMMYT
14	RHMM132^{+}	RML0005/RML0012//CML440	RAB	RAB	CIMMYT
15	RHMM129	RML0005/RML0011//CML488	RAB	RAB	CIMMYT
16	RHMM139^{++}	CML442/RML0011//CML445	RAB	RAB	CIMMYT
17	RHMM143^{+}	CML442/CML202//CML445	RAB	RAB	CIMMYT
18	RHMM126	RML0005/RML0011//CML440	RAB	RAB	CIMMYT
19	RHMM130^{++}	RML0005/RML0011//CML216	RAB	RAB	CIMMYT
20	RHM1403	CML444/CML442/CML216	CIMMYT	CIMMYT	CIMMYT
21	RHMM124	RML0005/RML0010//CML440	RAB	RAB	CIMMYT
22	RHMM150^{++}	RML0003/RML0006//CML216	RAB	RAB	CIMMYT
23	RHMM125^{+}	RML0005/RML0010//CML488	RAB	RAB	CIMMYT
24	RHMM137	RML0005/CML202//CML488	RAB	RAB	CIMMYT
25	RHM1407^{++}	CML539/CML444//CML445	CIMMYT	CIMMYT	CIMMYT
26	RHMM144	CML442/CML202//CML440	CIMMYT	CIMMYT	CIMMYT
27	RHMM120^{+}	RML0004/RML0012//CML440	RAB	RAB	CIMMYT
28	RHMM145^{+}	CML442/CML202//CML488	CIMMYT	CIMMYT	CIMMYT
29	RHMM111$^{+ǂ}$	RML0004/RML0010//CML445	RAB	RAB	CIMMYT
30	RHM1408	CML539/CML444/CML440	CIMMYT	CIMMYT	CIMMYT
31	RHM1406	CML536/CML489//CML216	CIMMYT	CIMMYT	CIMMYT
32	RHMM142^{++}	CML442/RML0011//CML216	CIMMYT	RAB	CIMMYT
33	RHMM122^{++}	RML0004/RML0012//CML216	RAB	RAB	CIMMYT
34	RHM1402^{++}	CML444/CML442//CML488	CIMMYT	CIMMYT	CIMMYT
35	RHM1401	CML444/CML442//CML440	CIMMYT	CIMMYT	CIMMYT
36	RHMM123^{+}	RML0005/RML0010//CML445	RAB	RAB	CIMMYT
37	RHMM140^{+}	CML442/RML0011//CML440	CIMMYT	RAB	CIMMYT
38	RHMM127^{++}	RML0005/RML0011//CML445	RAB	RAB	CIMMYT
39	RHMM131	RML0005/RML0012//CML445	RAB	RAB	CIMMYT
40	RHMM146^{+}	CML442/CML202//CML216	CIMMYT	CIMMYT	CIMMYT
41	RHMM141	CML442/RML0011//CML488	CIMMYT	RAB	CIMMYT

$^{+}$: Used under MLN natural inoculation
$^{++}$: Used in adaptability trials
CIMMYT: International Maize and Wheat Improvement Center
RAB: Rwanda Agriculture Board
SCH: Single Cross Hybrid
TWCH: Three Cross Hybrid

longitude 30°17'08''E, altitude 1372 masl). They included two Single Cross Hybrid (SCH) and 39 Three Way Cross Hybrid (TWCH) varieties (Table 1). The parent inbred lines from RAB were derived from three popular maize Open Pollinated Varieties (OPVs): Kigega (ZM607), Ndaruhutse (Pool 32) and ISARM101 (POP-NYA) through pedigree methodology as described by Ngaboyisonga *et al.* (2019). The parent inbred lines introduced from CIMMYT comprised CML442, CML444, CML445, CML202, and CML216 which are frequently used in Eastern Africa for hybrid combination (Table 2).

The 41 hybrid maize varieties were thereafter screened for MLN resistance under MLN artificial inoculation conditions at Naivasha (latitude 0°43′S, longitude 36°26′E, altitude 1896 masl) in Kenya. Trials were planted in Randomized Complete Block Design (RCBD) with two replications and a plot size of one row is 3 m length. Sowing was performed with two kernels per hill and next a thinning at one plant per hill reducing the plant stand to 13 plants per row. All the required agronomic practices were followed. The first inoculation with MLN was performed three weeks after planting.

The inoculum of MLN was obtained and maintained following the procedures of Gowda *et al.* (2015) by producing and maintaining separately the inocula of MCMV and SCMV. Briefly, original isolates of MCMV and SCMV were collected from MLN hotspot places and the confirmation of the presence of the two viruses was performed by Enzyme-Linked Immunosorbent Assay (ELISA). ELISA used, as directed by the supplier commercial kits, double antibody sandwich (DAS) type obtained from Agdia, Inc. (Agdia 2020). Then, the inocula of the two viruses were produced and maintained separately using MLN susceptible maize varieties. The two inocula were mixed with a ratio MCMV/SCMV of 1:4 for making the right MLN inoculum proportions. This proportion was directed by the fact that MCMV is very stable compared to SCMV. Furthermore, the combination of MCMV with SCMV was preferred because it was the mostly reported in Eastern Africa. Thereafter, the carborandum was added to the mixture with 1 g of carborandum in one litre of the mixture. The resulting inoculum (mixture of MCMV+SCMV+ carborandum) was kept in a cool environment till the inoculation was done.

The field trials were inoculated with MLN for the first time four weeks after planting when

Table 2: Characteristics of inbred lines used to generate the hybrid varieties used in the study

No	Names	Pedigree	Origin	Use in hybrid combination
1	CML202	ZSR923-B*4-5-1-B	CIMMYT	Frequently used
2	CML203	(7480TZVAR/TZSR)-Y-1-345-1-1-1-1-B*5-2-5-4-4-4-B	CIMMYT	Not frequently used
3	CML204	7794-4-1-B*9-1-4-7-4-5-B	CIMMYT	Not frequently used
4	CML216	MSR-131-3-3-3-5-B	CIMMYT	Frequently used
5	CML440	G16SEQ-C1-F47-2-1-2-1-B	CIMMYT	Not frequently used
6	CML442	(M37W/ZM607-#-B-F37SR-2-3SR-6-2-X)-8-2-X-1-B	CIMMYT	Frequently used
7	CML444	P43-C9-1-1-1-1-1-B	CIMMYT	Frequently used
8	CML445	(TUXPSEQ-C1-F2/P49SR)-F2-45-7-5-1-B	CIMMYT	Frequently used
9	CML488	DTPW-C8-F31-4-2-1-5-B	CIMMYT	Frequently used
10	CML539	MAS(MSR/CML312)-117-2-2-1-B	CIMMYT	Frequently used
11	RML0001	ZM607-76-3-1-B*4-#	RAB	Not yet used
12	RML0003	ZM607-38-1-1-B*4-#	RAB	Not yet used
13	RML0004	ZM607-34-2-1-B*4-#	RAB	Not yet used
14	RML0005	ZM607-80-4-1-B*4-#	RAB	Not yet used
15	RML0006	POOL32-70-2-1-B*4-#	RAB	Not yet used
16	RML0010	POOL32-76-2-1-B*4-#	RAB	Not yet used
17	RML0011	POOL32-6-3-1-B*4-#	RAB	Not yet used
18	RML0012	POOL32-11-4-1-B*4-#	RAB	Not yet used

the plants were at the 5-6 leaf stage. The second inoculation was carried out one week after the first. The inoculation was done using a motorized, backpack mist blower (Solo 423 MistBlower, 12 L capacity) following the procedures of Gowda *et al.* (2015). MLN symptoms appeared 10 to 15 days after the second inoculation depending upon the susceptible nature of the genotype. The MLN severity scoring was performed following the procedures of Gowda *et al.* (2015). It started two weeks after the second inoculation and was conducted for every two weeks for four consecutive steps. It was done at row basis and was rated visually on a 1 to 5 disease severity score, where 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. The genotypes with scores inferior to 2 were considered as resistant, those with scores between 2 and 2.5 moderately resistant, those with scores between 2.5 and 3.0 tolerant and those with scores superior to 3 susceptible. Furthermore, the Area Under Disease Progress Curve (AUDPC) was estimated as described by Simko and Piepho (2013) and Forbes *et al.* (2014) using the formulae:

$$AUDPC = \sum_{i=1}^{n} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i-1} - t_i)$$

where "y_i" was the value of the MLN score at "i^{th}" scoring step , "t_i" the time at "i^{th}" scoring step and "n" is the total number of scoring steps. The time of scoring "t_i" was recorded in the number of days after the second inoculation.

The AUDPC was used to determine the relative AUDPC noted rAUDPC. The rAUDPC was estimated by dividing the AUDPC of each variety with the maximum potential AUDPC by the formulae: $rAUDPC = \frac{AUDPC}{(t_n - t_1)\times 5}$.

The maximum potential AUDPC was the AUDPC a variety would have if it had MLN score of 5 at all scoring steps. The analysis of data was performed using Genstat 20th Edition following the procedures of Baird *et al.* (2019).

Evaluation under MLN natural inoculation

Twenty-four hybrid maize varieties (Table 1) based on their performance under MLN artificial inoculation were used in the evaluation trials under MLN natural inoculation. The trials were conducted at Karama research station (latitude 2°16'12''S, longitude 30°15'37''E, altitude 1339 masl) in 2017 A (October 2016-February 2017) and 2017 B seasons (March-July 2017). The experimental design was an alpha lattice (8x3) with four replications. The plot size was two rows of 5-m length. Planting was performed at the spacing of 0.75 m between rows and 0.25 m between hills. Sowing was done by two kernels per hill and a thinning two weeks later reduced the plant stand at one seedling per hill. All usual agronomic practices including mineral fertilizers application and weeding were applied.

Data recording included first and second MLN severity scoring, ear rot, grain yield at 15 % grain moisture content, and AUDPC. The first scoring was done at 69 days after planting at the blister stage whereas the second scoring was performed 113 days after planting before physiological maturity when the leaves were still green which was also 44 days after the first rating. The scoring of MLN severity was performed following the procedures of Gowda *et al.* (2015).

The ear rot was obtained in percentage by taking the number of rotten ears divided by the number of harvested ears. Grain yield was obtained by weighing the total ears harvested (fresh weight in kg, FW) and sampling kernels to obtain grain moisture (GM in %) using a portable moisture meter. Grain yield (GY) in t/ha at 15 % of grain moisture content was calculated by taking A as the distance (in m) between rows, B as the distance (in m) between hills at planting, C as the length (in m) of harvested rows, D as the number of rows harvested, DW as the dry weight (in kg) after drying the ears, and GW as the grain weight (in kg) obtained after shelling. using following equation:

$$GY = \frac{FW \times 10}{A(B+C)D} \times \frac{100 - GM}{100 - 15} \times \frac{GW}{DW}$$

Additionally, the AUDPC and rAUDC were calculated following the procedures of Forbes *et al*. (2014). The analysis of data was performed by using Genstat 20th Edition following the procedures of Baird *et al*. (2019) and considering the experimental design as Randomized Complete Block Design (RCBD).

Evaluation of 12 elite hybrid maize varieties in multi-environmental trials

Twelve selected elite hybrid maize varieties (Table 1) based on their performance under MLN natural inoculation were evaluated at four sites comprising Cyabayaga, Karama, Rubona, and Bugarama (Table 3) both in the 2018 A season (October 2017-February 2018) and 2018 B season (February-July 2018), hence making eight evaluation environments (site × season).

Rubona site is the coolest and the highest in altitude and receives, on average 1,171 mm rain per year. In this site, the incidence of diseases on maize is moderate and drought occurs occasionally. The Bugarama site has the lowest altitude in the country. It is the hottest of the sites considered a hot spot for maize streak virus disease. The site experiences drought occasionally. Cyabayaga site is the hot spot of turcicum leaf blight and grey leaf spot diseases. At Karama, the drought is very frequent and more severe than in other sites. All four sites have two cropping seasons in a year that overlap in February. According to Henninger (2013), Rubona is situated in the temperate zone of the central highlands, Karama and Cyabayga in East Rwandan, dry and hot lowland while Bugarama is located in the Lake Kivu climate where the prevailing land-lake-wind circulation creates better climatic conditions.

The experimental design was alpha-lattice (4×3) with three replications, and analyzed as RCBD. A plot was made by three rows of 5-m length with a distance between rows of 0.75 m and a distance between hills of 0.25 m. Planting was performed by two grains per hill followed by a thinning at one plant/hill three weeks after planting. Fertilizers were applied at rates of 51 kg/ha N, 51 kg/ha P_2O5 and 51 kg/ha K_2O before planting. Six weeks after planting, 46 kg/ha N using urea (46-0-0) were applied at a rate of 100 kg/ha. Rainfall served as the primary source of water, and weeding was done as it was needed.

Grain yield (t/ha at 15 % grain moisture) was the trait recorded in all trials. The AMMI (Additive Main effects and Multiplicative Interactions) model was used to analyze the Variety × Environment Interaction (VEI). The AMMI analysis of variance was performed using Genstat 20th Edition following the procedures of Baird *et al.* (2019) whereas AMMI1 biplots were constructed using the MS Excel spreadsheet. The stability of varieties was obtained by the yield stability index (YSI) determined by the ranks of AMMI stability values (ASVs) and grain yields as described by Farshadfar *et al.* (2011) and Oliveira *et al.* (2013). The ASVs were obtained using the formulae:

Table 3: Characteristics of evaluation sites

Site name	Altitude (masl)	Rain (mm/ year)	Latitude	Longitude	Climate (Henninger, 2013)	Constraints
Cyabayaga	1372	850	1°24’25’’S	30°17’08’’E	East Rwandan, dry and hot lowland	Hot spot of Turcicum Leaf Blight (TLB) and Grey Leaf Spot (GLS) diseases
Karama	1339	830	2°16’12’’S	30°15’37’’E	East Rwandan, dry and hot lowland	Frequent drought occur-rence
Rubona	1691	1170	2°28’55’’S	29°00’37’’E	Temperate zone of the central high-lands	-
Bugarama	1055	1000	2°38’37’’S	29°00’36’’E	Kivu Lake climate	Hot spot of Maize Streak Virus (MSV) disease

ASV=

$$\sqrt{\left[\frac{IPCA1_{SS}}{IPCA2_{SS}} \times (IPCA1_{Score})\right]^2 + (IPCA2_{score})^2}$$

where ASV is the AMMI stability value, $IPCA1_{SS}$ is the sums squares of IPCA1 in the AMMI analysis of variance, $IPCA2_{SS}$ is the sums squares of IPCA2 in the AMMI analysis of variance, $IPCA1_{Score}$ is the IPCA1 score and $IPCA2_{Score}$ is the IPCA2 score. The varieties were ranked in ascending order using ASVs because an ASV close to zero indicates high stability. Furthermore, using grain yields, the varieties were ranked in descending order. The two ranks were added to obtain the YSI.

RESULTS AND DISCUSSION

Screening under MLN artificial inoculation

The availability of MLN artificial inoculation facilities has allowed making progress in breeding for MLN resistance and has speeded the process of developing MLN tolerant or resistant varieties in Eastern and Central (Gowda *et al.* 2015; Boddupalli *et al.* 2020) although the MLN disease was new in Africa (Wangai *et al.* 2012). The disease is still a serious threat to food security in Sub-Saharan Africa especially in the regions where maize is the staple food crop (Mahuku *et al*. 2015a). It is anticipated that the current scenario would continue to worsen, causing further damage. (Isabirwhye, and Rwomushana 2016). The utilization of MLN-resistant varieties represents an economically viable, environmentally sustainable approach and a durable measure to control the MLN disease (Mahuku *et al.* 2015a).

The results of the analysis of variance showed significant differences between varieties for the first (P=0.001) and the second (P=0.017) MLN scorings and non-significant differences between varieties for the third (P=0.100) and the fourth (P=0.398) MLN scorings (Table 4). Furthermore, the differences between varieties were highly significant (P<0.001) for the AUDPC and rAUDPC.

The first MLN scoring varied from 1.3 (RHT132P) to 2.8 (RHMM129) with an average of 2.1. The scores of the second MLN changed from 2.0 (RHM1409) to 4.0 (RHMM128) with 3.1 on average. The third MLN scoring varied from 2.5 (RHM1409) to 4.0 (RHMM128, RHM104P) with an average of 3.1 while the fourth increased from 2.5 to 4.0 with a value of 3.3 on average. The AUDPC increased from 92.8 (RHM1409) to 157.5 (RHMM128) with a mean of 124.4 whereas the rAUDPC changed from 0.44 (RHM1409) to 0.75 (RHMM128) with a mean of 0.59 (Table 4).

Twenty-two varieties had the first MLN scoring values inferior or equal to 2.0 whereas eleven varieties had the second MLN scoring values inferior or equal to 2.8. Ten varieties comprising RHM1409 (2.5), RHT132P (2.5), RHT132 (2.5), RHMM132 (2.5), RHMM123 (2.5), RHMM140 (2.8), RHM1407 (2.8), RHMM113 (2.8), RHMM125 (2.8) and RHM1402 (2.8) had the third MLN scoring values inferior or equal to 2.8 while eight varieties comprising RHM1409 (2.5), RHT132P (2.8), RHT132 (2.8), RHMM132 (2.8), RHMM140 (2.8), RHMM123 (2.8), RHM1407 (2.9) and RHM1402 (2.9) had the fourth MLN scoring values inferior to 3.0 (Table 4).

Twenty-five varieties that included 24 TWCHs and one SCH had a rAUDPC inferior or equal to 0.60 whereas only six varieties comprising RHM1409 (0.44), RHT132P (0.45), RHT132 (0.46), RHMM132 (0.48), RHMM123 (0.49) and RHMM140 (0.49) had a rAUDPC inferior to 0.50. The 24 TWCH varieties with rAUDPC inferior or equal to 0.60 or the third MLN scoring values inferior or equal to 3.0 had an acceptable level of tolerance to MLN (Table 4).

The strategy of screening 41 hybrid maize varieties under artificial inoculation in the first step had the advantages of identifying 24 varieties having acceptable levels of MLN tolerance with an rAUDPC inferior or equal to 0.60. A small AUDPC or a small rAUDPC closer to zero indicates low susceptibility to the disease whereas a high AUDPC or a rAUDPC close to 1 implies a very high susceptibility to the disease (Paraschivu *et al.*

2013). Hence, the varieties: RHM1409, RHT132P, RHT132, RHMM132 RHMM140 and RHMM123 were MLN moderately resistant with a rAUDPC inferior to 0.50 whereas the varieties RHM1407, RHMM113, RHM1402, and RHMM125 were MLN tolerant with a rAUDPC inferior to 0.55.

The AUDPC and its derivative rAUDPC, and the standardized AUDPC noted sAUDPC (Simko and Piepho 2012) have been used to correctly measure the level of susceptibility to diseases in various crops (Mukherjee *et al.* 2010; Forbes *et al.* 2014; Kebede and Golla 2020) including MLN (Karanja *et al.* 2018). They have been found to be specifically useful in the identification of MLN-resistant maize germplasm (Karanja *et al.* 2018; Nyaga *et al.* 2020).

Table 4: Performance of 41 maize hybrid varieties under MLN artificial inoculation

No	Names	MLN scoring 1	MLN scoring 2	MLN Scoring 3	MLN scoring 4	AUDPC	rAUDPC
1	RHT132P	1.3	2.3	2.5	2.8	94.5	0.45
2	RHM1409+	1.8	2.0	2.5	2.5	92.8	0.44
3	RHT132+	1.5	2.3	2.5	2.8	96.3	0.46
4	RHMM132+	2.0	2.3	2.5	2.8	99.8	0.48
5	RHMM123+	2.0	2.5	2.5	2.8	103.3	0.49
6	RHMM140+	2.5	2.3	2.5	2.8	103.3	0.49
7	RHM1407+	1.5	2.8	2.8	2.9	107.8	0.51
8	RHMM113+	2.0	2.6	2.8	3.0	109.2	0.52
9	RHM1402+	2.2	2.6	2.8	2.9	110.6	0.53
10	RHMM125+	2.5	2.8	2.8	3.0	115.5	0.55
11	RHT131+	2.0	3.0	2.9	3.0	116.9	0.56
12	RHMM150+	2.0	2.8	3.0	3.0	115.5	0.55
13	RHMM128+	2.0	3.0	3.0	3.0	119.0	0.57
14	RHMM139+	1.5	3.0	3.0	3.5	119.0	0.57
15	RHMM143+	1.5	3.0	3.0	3.5	119.0	0.57
16	RHMM127+	2.3	3.0	3.0	3.0	120.8	0.58
17	RHMM120+	2.0	3.0	3.0	3.5	122.5	0.58
18	RHMM145+	2.5	3.0	3.0	3.0	122.5	0.58
19	RHMM111+	2.0	3.0	3.0	3.5	122.5	0.58
20	RHMM146+	2.5	3.0	3.0	3.0	122.5	0.58
21	RHMM124	2.5	3.5	3.5	3.5	140.0	0.67
22	RHMM115+	2.5	3.0	3.0	3.5	126.0	0.60
23	RHMM130+	2.0	3.0	3.0	3.5	122.5	0.58
24	RHMM142+	2.0	3.0	3.0	3.5	122.5	0.58
25	RHMM129	2.8	3.5	3.5	3.5	141.8	0.68
26	RHMM137	2.5	3.5	3.5	3.5	140.0	0.67
27	RHMM122+	2.0	3.0	3.0	4.0	126.0	0.60
28	RHM104+	1.5	3.0	3.5	3.5	126.0	0.60
29	RHM102	1.3	3.5	3.5	4.0	134.8	0.64
30	RHM1401	2.0	3.5	3.5	3.5	136.5	0.65
31	RHMM144	2.0	3.5	3.5	3.5	136.5	0.65
32	RHMM119	2.1	3.5	3.5	3.5	136.9	0.65
33	RHM1403	2.0	3.5	3.5	4.0	140.0	0.67
34	RHM1408	2.5	3.5	3.5	3.5	140.0	0.67
35	RHM1406	2.5	3.5	3.5	3.5	140.0	0.67
36	RHMM121	2.5	3.5	3.5	3.5	140.0	0.67
37	RHMM131	2.5	3.5	3.5	3.5	140.0	0.67
38	RHMM141	2.5	3.5	3.5	3.5	140.0	0.67
39	RHM1405	2.5	3.5	3.5	4.0	143.5	0.68
40	RHT104P	2.3	3.5	4.0	4.0	148.8	0.71
41	RHMM128	2.5	4.0	4.0	4.0	157.5	0.75
Means		2.1	3.1	3.1	3.3	124.7	0.59
C.V. (%)		22.0	18.7	16.7	17.0	8.2	8.2
F		2.61	1.96	1.50	1.08	4.54	4.54
P		0.001	0.017	0.100	0.398	<.001	<.001

+: Three Way Cross Hybrid varieties having satisfactory level of tolerance to MLN

Evaluation under MLN natural inoculation

The screening of selected varieties under MLN natural inoculation had the purpose of knowing their performance under farmers' similar conditions although the expression of MLN symptoms may depend on several uncontrolled factors (Jain *et al.* 2019).

The analysis of variance showed significant differences between varieties for the first MLN scoring, the second MLN scoring, AUDPC, rAUDPC, ear rot, and the grain yield at P<0.001. The first MLN scoring varied from 1.1 (RHMM1402) to 2.4 (RHMM125) with an average of 1.8 whereas the second MLN scoring varied from 1.8 (RHM1402) to 3.8 (RHMM125) with an average of 2.6. The rAUDPC varied from 0.28 (RHM1402) to 0.61 (RHMM125) with an average of 0.45. The ear rot varied from 30.1 % (RHM1402) to 81.9 % (RHM104) with an average of 54.4 % while the grain yield changed from 1.20 t/ha (RHMM125) to 4.17 t/ha (RHM1402) with an average of 2.41 t/ha (Table 5).

Sixteen varieties had the first MLN scoring values inferior to 2.0 whereas 12 varieties comprising RHM1402, RHM1409, RHM1407, RHT132, RHMM142, RHMM150, RHMM122, RHT131, RHMM139, RHMM111, RHMM127 and RHMM113 had second MLN scoring values inferior to 2.5. Furthermore, eleven varieties including RHM1402, RHM1409, RHM1407, RHMM111, RHT132, RHMM127, RHMM142, RHMM122, RHMM150, RHMM130 and RHMM113 had rAUDPC values inferior to 0.43 while eight varieties comprising RHM1402, RHM1409, RHMM130, RHT132, RHMM142, RHMM122, RHM1407 and RHT131 had ear rot incidence inferior to 45 %. Additionally, eight varieties including: RHM1402, RHM1409, RHMM130, RHT132, RHMM142, RHMM122, RHM1407 and RHT131 had grain yields superior to 3.0 t/ha. Twelve varieties comprising RHM1402,

Table 5: Performance of 24 hybrid varieties under natural MLN infestation

No	Names	MLN scoring 1	MLN scoring 2	AUDPC	rAUDPC	Ear rot (%)	Grain yield (t/ha @15 % H_2O)
1	RHMM130	1.6	2.6	33.3	2.80	90.8	0.41
2	RHT132	1.8	2.1	33.7	3.29	86.6	0.39
3	RHMM142	1.9	2.1	36.4	3.44	88.0	0.40
4	RHMM127	1.6	2.4	49.2	2.29	86.6	0.39
5	RHM1409	1.3	1.8	32.5	3.52	66.0	0.30
6	RHMM139	2.1	2.3	57.7	2.66	94.9	0.43
7	RHM1402	1.1	1.8	30.1	4.17	61.9	0.28
8	RHM1407	1.4	1.8	41.7	3.30	71.5	0.33
9	RHMM122	1.8	2.3	40.1	3.22	88.0	0.40
10	RHMM150	1.8	2.3	51.7	2.33	88.0	0.40
11	RHMM125	2.4	3.8	77.6	1.20	134.8	0.61
12	RHMM113	1.8	2.4	48.8	2.40	92.1	0.42
13	RHMM111	1.5	2.4	49.2	3.03	85.2	0.39
14	RHMM143	2.2	3.6	76.1	1.64	127.9	0.58
15	RHMM115	2.0	2.8	59.9	2.34	105.9	0.48
16	RHMM123	1.8	3.4	71.8	1.72	114.1	0.52
17	RHMM120	1.8	2.6	58.5	2.13	97.6	0.44
18	RHMM132	1.9	2.6	54.4	1.74	99.0	0.45
19	RHMM128	2.1	2.8	58	1.81	107.2	0.49
20	RHT131	2.1	2.3	44.9	2.37	94.9	0.43
21	RHMM145	1.9	3.5	81.3	1.37	119.3	0.54
22	RHMM146	1.5	3.4	70.3	1.53	108.6	0.49
23	RHMM140	2.3	3.2	66.5	2.02	119.6	0.54
24	RHM104	2.2	3.4	81.9	1.44	122.4	0.56
Means		1.8	2.6	54.4	2.41	98.0	0.45
C.V. (%)		19.2	24.3	27.6	34.5	17.2	17.20
F		6.85	7.26	9.37	7.37	9.98	9.98
P		<.001	<.001	<.001	<.001	<.001	<.001

RHM1409, RHM1407, RHMM111, RHT132, RHMM127, RHMM142, RHMM122, RHMM150, RHMM130, RHMM113 with rAUDPC inferior to 45 % had satisfactory tolerance level to MLN disease. The varieties RHM1409, RHM1402, and RHM1407 with second MLN scoring values of 1.8 and rAUDPC values inferior to 0.34 were moderately resistant to MLN disease (Table 5).

The slope of the regression of rAUDPC on the ear rot (b=159.89) was positive and significant at P<0.001. On the contrary, the coefficient of regression of rAUDPC on grain yield (b=-8.20) was negative and significant at P<0.001 (Figure 1).

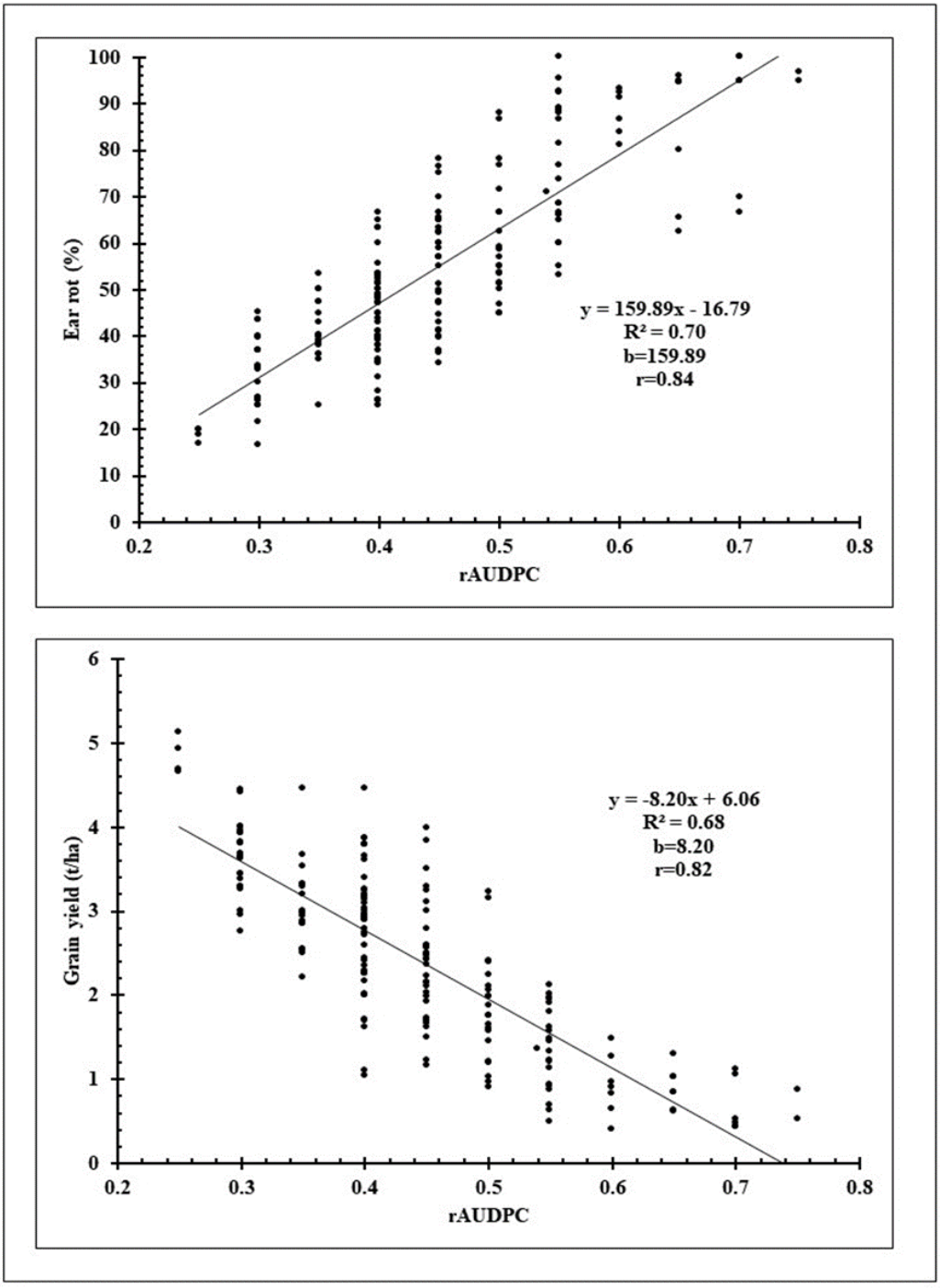

Figure 1: Regression of rAUDPC on ear rot and grain yield

The positive and significant slope of the regression between rAUDPC and ear rot suggests that MLN disease causes and aggravate maize ear rot. The increase in ear rot incidence of maize may have resulted from the thrips, vector of MCMV, while feeding on immature grains as suggested by Redinbaugh and Stewart (2018). The maize ear rot may be associated with mycotoxins including the most dangerous aflatoxins (Ogara *et al.* 2017; Logrieco *et al.* 2021). Consequently, their increase may augment the risks of such aflatoxins thus, may pose health concerns (Fung and Clark 2004; Kumar *et al.* 2017; Imran *et al.* 2020). Furthermore, a negative and significant slope of the regression between rAUDPC and grain yield implies that the MLN incidence reduces the grain yields of varieties causing serious impact on maize production (Marenya *et al.* 2018). According to de Groote *et al.* (2016) maize losses in affected areas of Kenya in 2012-2013 varied from 23 % to 100 %.

The first MLN scoring was done 69 days after planting at the blister stage whereas the second MLN scoring was performed at 113 days before physiological maturity when the MLN symptoms were fully expressed making the scoring to be executed in two points in the time. Hence, the AUDPC and rAUDPC were estimated based on two data points in the time. Mukherjee *et al.* (2010) used two data recording points, the first and the last data points on one hand and several data points on the other hand, to estimate the AUDPC of rice blast in order to find similar results given by the two estimation procedures.

The strategy of screening 24 varieties having adequate levels of MLN tolerance under natural inoculation permitted the identification of 11 varieties (RHM1402, RHM1409, RHM1407, RHMM111, RHT132, RHMM127, RHMM142, RHMM122, RHMM150, RHMM130 and RHMM113) with satisfactory MLN tolerance having a rAUDPC inferior to 0.45.

Evaluation of 12 elite hybrid maize varieties in multi-environmental trials

The AMMI analysis of variance showed that the variation due to varieties, environments and VEI was highly significant ($p<0.001$) (Table 6). The variety effect accounted for 37.6 % of the treatment sums squares followed by environmental effects (40.7 %) and VEI effects (21.7 %) accounted for the remaining percentage. The treatment sums squares were obtained by the addition of variety sums squares with environment sums square and VEI sums squares. Therefore, the percentage of effects is the proportion accounted for in the treatment sums square. The variation due to environments was slightly higher than the variation due to varieties whereas it was approximately two times larger than the VEI variation. However, both variety and VEI variations accounted for 59.3 % which was larger than the environmental variation. Moreover, the variation due to varieties was 1.7 times larger

Table 6: AMMI analysis of variance of 12 varieties evaluated in eight environments

Sources of variation	DF	SS	MS	F	P
Total	287	1072.3	3.74	-	-
Treatments	95	958.0	10.08	18.16	<0.001
Varieties	11	360.3	32.75	58.99	<0.001
Environments	7	389.7	55.67	53.61	<0.001
Environments/Replications	16	16.6	1.04	1.87	0.026
Varieties × Environments	77	208.0	2.70	4.87	<0.001
IPCA1	17	85.0	5.00	9.00	<0.001
IPCA2	15	62.9	4.19	7.55	<0.001
IPCA3	13	34.5	2.65	4.78	<0.001
IPCA4	11	13.1	1.19	2.15	0.019
Residuals	21	12.5	0.60	1.08	0.378
Error	176	97.7	0.56	-	-

than the VEI variation. The AMMI analysis of variance further showed that the first four IPCAs were significant at P≤0.019. IPCA1 axis captured 40.9 % of the VEI sums of squares, IPCA2 explained 30.2 % of the VEI sums of squares while both IPCA1 and IPCA2 captured 71.1 % of the VEI sums of squares.

In AMMI analysis of variance, the treatment variation is subdivided into three types of variations: variation due to genotypes main effects, variation due to environments main effects, and variation due to Genotype × Environment Interaction (GEI) effects. These three sources of variation present different problems and opportunities. The genotype variation pertains to broad adaptations, the GEI variation is related to narrow adaptations, while genotypes and GEI variations jointly determine mega-environments (Gauch 2006; Gauch *et al.* 2008; Hongyu *et al.* 2014). In the present study, the variation due to varieties jointly with the VEI variation was larger than the environmental variation implying that mega-environments effects were far important than the effects of individual environments. Moreover, the broad adaptation was very larger than the narrow adaptation indicating that the varieties tended to be broadly adapted. Broad adaptation implies that varieties tend to be adapted in several and diverse environments whereas narrow adaptation is when varieties tend to be adapted in specific environments (Gauch 2013). Likewise, there are several studies where it was found that mega-environment effects were more important than the effects of individual environments and broad adaptation is higher than narrow adaptation (Mukherjee *et al.* 2013; Ngaboyisonga *et al.* 2014). Also, studies where environment variation was the most important than the two other components were frequently reported (Beyene *et al.* 2011).

The AMM1 biplot showed that the varieties: RHM1402, RHM1409, RHMM113,

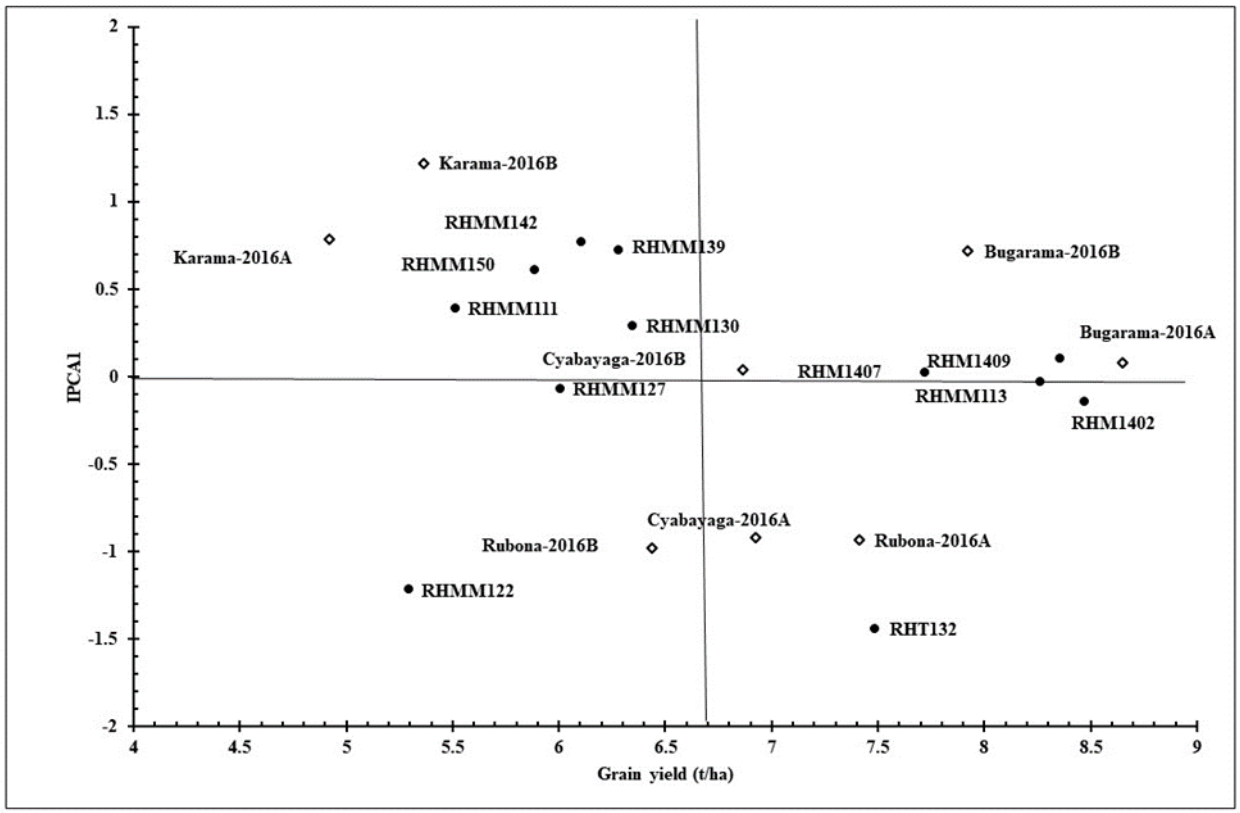

Figure 2: Biplot of grain yield obtained by plotting the means (t/ha) against IPCA1 [(t/ha)$^{0.5}$] for 12 varieties evaluated in eight environments

RHM1407 and RHT132 had grain yields superior to overall mean of 6.8 t/ha. The remaining varieties had grain yields inferior to the overall mean. Furthermore, the cluster made by the varieties RHM107, RHM113, RHM1409 and RHM1402 was very close to the abscissa while the variety RHT132 was very far from it. The varieties, RHM1407, RHM113, RHM1409, and RHM1402 made a cluster with Bugarama-2018 A and Bugarama 2018 B environments whereas RHT132 was very close to the Rubona-2018 A environment. Bugarama-2018A environment had the highest grain yield of 8.7 t/ha and Karama-2018 A had the lowest grain yield of 3.9 t/ha (Figure 2).

In AMMI 1 biplot, the usual interpretation of a biplot is that displacements along the abscissa indicate differences in main (additive) effects, whereas displacements along the ordinate indicate differences in interaction effects. Varieties that group together have similar adaptation while environments that group together influences the genotypes in the same way. When a variety and an environment have the same sign on the IPCA axis, their interaction is positive and if the sign is opposite, their interaction is negative. If a variety has a high mean (mean > overall mean) and an IPCA1 score closer to zero (near the abscissa), it has small interaction effects and it is considered stable across environments (Neisse *et al.* 2018). Therefore, the varieties RHM102, RHM1402, RHM1409, RHMM113, and RHM1407 had similar adaptation and were stable across environments because they have high means and were very close to the abscissa. Additionally, they interacted positively with high productive environments. The variety RHT132 was not stable across environments although it had a high mean. However, it was particularly adapted to specific environments, Rubona-2016 A and Cyabayaga-2016 A.

The ranking of varieties showed that the six first varieties arranged in ascending order by the ASVs comprised; RHM1407, RHMM113, RHMM127, RHM1409, RHMM130, and RHM1402 whereas six varieties arranged in descending order by the grain yield included; RHM1402, RHM1409, RHMM113, RHM1407, RHT132, and RHMM130. Therefore, four varieties with excellent YSI inferior to 10 were RHMM113 (5), RHM1407 (5), RHM1409 (6), and RHM1402 (7) (Table 7).

The YSI and or the ASVs have been used to identify superior genotypes in several crops (Farshadfar *et al.* 2011; Oliveira *et al*. 2013; Kumar *et al*. 2018) including maize (Oyekunle *et al.* 2017; Katsenios *et al*. 2021).

Table 7: Ranking of 12 varieties evaluated in eight environments based on AMMI stability values and yield index

No	Variety	Grain yield (t/ha)	IP-CA1	IPCA2	ASV	Yield rank	ASV rank	YSI
1	RHM1407	7.723	0.024	-0.700	0.023	4	1	5
2	RHMM113	8.263	-0.030	0.618	0.025	3	2	5
3	RHM1409	8.357	0.104	0.388	0.055	2	4	6
4	RHM1402	8.471	-0.144	0.846	0.165	1	6	7
5	RHMM130	6.349	0.289	0.344	0.134	6	5	11
6	RHMM127	6.008	-0.069	-0.351	0.033	9	3	12
7	RHMM142	6.104	0.767	-0.628	0.651	8	9	17
8	RHT132	7.487	-1.443	-0.623	1.215	5	12	17
9	RHMM111	5.514	0.387	-0.604	0.316	11	7	18
10	RHMM150	5.889	0.612	-0.632	0.523	10	8	18
11	RHMM139	6.283	0.722	0.945	0.921	7	11	18
12	RHMM122	5.296	-1.219	0.398	0.656	12	10	22

The use of YSI, in this study, has allowed identifying the varieties: RHMM113, RHM1407, RHM1409, and RHM1402 having excellent performance (YSI<10). The varieties RHM113, RHM1407, RHM1409, and RHM1402 were identified as superior genotypes by combing the AMM1 biplot and YSI.

CONCLUSIONS

The identification process of tolerant or resistant maize varieties with high yield and stable across environments was conducted in three steps: screening under MLN artificial inoculation, evaluation under MLN natural inoculation, and multi-environments evaluations.

Three varieties comprising RHM1409, RHM1402, and RHM1407 had substantial performance under both artificial and natural MLN inoculations and were classified as MLN moderately resistant cultivars. Furthermore, the AMMI analysis and the YSI showed that the varieties RHM113, RHM1409, RHM1407, and RHM1402 were high yielding and stable across tested environments. By combing MLN artificial and natural inoculations with AMMI analysis and YSI, three varieties, RHM1409, RHM1407, and RHM1402 were shown MLN moderately resistance, high yielding, and stable across environments. These four varieties are proposed to be released to farmers and to be integrated with other control strategies to manage MLN disease in Rwanda.

The study has further shown that MLN infection not only reduced the grain production, but also increased the incidence of maize ear rots, which may be attributed to mycotoxins, particularly aflatoxins.

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AUTHOR CONTRIBUTION

CN designed the study plan and performed the data analysis and interpretation. He wrote the first manuscript of the paper. All authors participated in the implementing the study and editing the manuscript. They consented to its publication.

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