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Evaluation of maize germplasm for resistance to maize chlorotic mottle virus and sugarcane mosaic virus: The casual agents of maize lethal necrosis disease

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In Kenya, at national policy level to the individual household level, food security is synonymous to maize productivity and availability. However, the productivity of maize is affected majorly by maize lethal necrosis disease (MLND) that was first reported in Kenya in 2011. MLND results from co-infection between maize chlorotic mottle virus (MCMV) and any cereal-infecting viruses in Potyviridae family particularly sugarcane mosaic virus (SCMV). Majority of maize germplasm are susceptible to MLND. This study was therefore carried out to identify potential germplasm for breeding for MLND resistance. A total of 38 maize germplasm (5 temperate lines with inherent resistance to maize-infecting viral diseases, 32 assorted tropical lines and one Kenyan hybrid) were artificially inoculated with MCMV and SCMV in the green house at the University of Nairobi Field Station and screened for two seasons between April 2020 and October 2021. Based on the Area Under Disease Progress Curve (AUDPC) and final severity score, germplasm KS23-6, 18, KS23-5 and 19 were identified as the most promising sources of MCMV resistance with disease severity scores of 2, 2.3, 2.3 and 3, respectively while germplasm 50, 19, and 22 were identified as source of SCMV resistance with scores of 2.0, 2.3 and 3, respectively. These germplasms could serve as potential donors for introgression of the resistance genes into locally adapted maize background to combat yield losses due to MLND.

Key words: Maize lethal necrosis disease, sugarcane mosaic virus, maize chlorotic mottle virus, resistance, maize germplasm.

INTRODUCTION

Maize (*Zea mays*) contributes significantly to food security in Kenya; with 90% of the country population depending on maize as the main staple food and source of income (Eunice et al., 2021). Per capita consumption of maize in Kenya is between 98 to 100 kg (Onono et al., 2013). Maize occupies 2.1 million ha which is 40% of the total crop area and the annual yield was 3.39 million tons in 2016 (Mwatuni et al., 2020) and 3.8 million tons in 2021

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(FAOSTAT, 2022). Maize production suffers from abiotic and biotic stress. Abiotic stresses include low rainfall and infertile soils (Simtowe et al., 2020) while biotic stress include diseases and insect pest such as aphids, thrips and fall armyworm (De Groote et al., 2020). Diseases such as Gray leaf spot, Common smut, Northern leaf blight, Maize streak virus and Head smut are endemic in major maize growing regions (Charles et al., 2019). Emergence of maize lethal necrosis disease (MLND) in 2011 saw the most devastating effect on maize production in Kenya (Wangai et al., 2011; Marenya et al., 2018; Jafari et al., 2020; Redinbaugh and Stewart, 2018; Wamaitha et al., 2018). MLND is commonly caused by synergistic interaction between maize chlorotic mottle virus (MCMV) and sugarcane mosaic virus (SCMV) (Adams et al., 2014; Mwatuni et al., 2020). In addition, other potyviruses such as maize dwarf mosaic virus (MDMV), wheat streak mosaic virus (WSMV) (Mahuku et al., 2015; Masanga et al., 2020), and Johnson grass mosaic virus (JGMV) (Stewart et al., 2017) can associate with MCMV to induce MLND. Symptoms associated with MLND and its causative viruses include chlorotic specks on young leaves, leave necrosis, shortening of internodes, premature dying of the husks and few grains filling at maturity stage (Mahuku et al., 2015).

MCMV is the only member in genus *Machlomovirus* of the family Tombusviridae (Zhang et al., 2011). It has been reported in Peru (Nault et al., 1978), USA, Argentina, Brazil (Braidwood et al., 2018), and China (Wang et al., 2017). In Kenya, MCMV was first reported in Bomet (Wangai et al., 2012) and later in all maize growing regions of the country. Presence of MCMV in the region resulted in the outbreak of devastating MLND leading to almost 100% yield losses of maize (Lukanda et al., 2014; Adams et al., 2012). MCMV is transmitted by onion thrips (*Thrips tabaci*), maize thrips (*Frankliniella williamsi*) (Mwando et al., 2018), and at least six beetle species (Isabirye and Rwomushana, 2016) and through seed but at very low rate (Kimani et al., 2021; Jensen, 1991).

SCMV is more prevalent worldwide (Masanga et al., 2020) and was first reported in USA in 1963 (Janson and Ellet, 1963). A study by Louie (1980) confirmed presence of SCMV in 20 of 33 districts surveyed in Kenya. It belongs to *Potyvirus* genus of Potyviridae family (Redinaugh and Stewart, 2018). SCMV is transmitted by aphids (Redinaugh and Stewart, 2018).

Management strategies for MLND include crop nutrition, weed control (Fatma et al., 2016), crop rotation (Frank et al., 2016), and use of certified seeds (Mwatuni et al., 2020). However, it is difficult to manage MLND using these strategies due to nature of its spread (Mudde et al., 2018). Breeding for resistance is the most effective and sustainable method to manage MLND (Beyeni et al., 2017; Awata et al., 2021). This study focused on identifying germplasm that are resistant to maize lethal necrosis disease causative viruses (MCMV and SCMV) that can be used as donor in breeding programs.

MATERIALS AND METHODS

Plant, experimental site and layout

A total of 38 maize germplasm (5 temperate lines with inherent resistance to maize-infecting viral diseases 32 assorted tropical lines and 1 Kenyan hybrid) were evaluated (Table 1). The experiment was conducted in a net house at the University of Nairobi, College of Agriculture and Veterinary Sciences' field station. The station is situated in Kabete, which lies at a longitude of 36° 44" East and latitude of 1° 15" South and about 1940 m above the sea level. The area experiences a bimodal rainfall averaging 1000 mm of rainfall per annum. The site's daily maximum temperature ranges between 13 and 27°C (Wasonga et al., 2015). Maize germplasms were screened for their responses to MCMV and SCMV for two seasons in 2020 and 2021. Completely randomized design (CRD) was used to set up the experiments with three replications. Three maize seeds per pot were planted in black polythene pots measuring 30 cm diameter and 30 cm height. Di-ammonium Phosphate (DAP) was applied at planting 5 g per pot. Watering was done four times a week.

Preparation of the virus inoculum and leaf inoculation

At three leave stage, maize seedlings were singly inoculated with MCMV and SCMV, respectively as described by Karanja et al. (2018) and Sitta et al. (2017). The inoculum was prepared from maize leaves showing classical MCMV and SCMV symptoms derived from virus collection at Kenya Agricultural and Livestock Research Organization (KALRO), Biotechnology center. Inoculation solution (0.1 M phosphate buffer) was constituted by dissolving 10.8 g of potassium phosphate monobasic, 4.8 g potassium phosphate dibasic, 1.26 g Na_2SO_3 and 1 g of Carborandum in 1 L of sterile distilled water (Sitta et al., 2017). Reagents were from SIGMA® Life Science. For each of the virus isolate, 200 g of infected leaves were obtained, homogenized and dissolved in 1 L of the inoculum buffer. The inoculum was applied on the leaves by hand rubbing. A second inoculation was done one week later (Tembo et al., 2021).

Data collection/rating

Data was collected on disease severity as described by International Maize and Wheat Improvement Center (CIMMYT). Disease severity was based on visual subjective five-point scale of 1-5, where 5 represent very severe symptoms, 4 severe symptoms, 3 moderate symptoms, 2 mild symptoms and 1 no symptoms (Figure 1) (Karanja et al., 2018; Sitta et al., 2017). Data was collected for six weeks after the first inoculation.

Data analysis

Analysis of variance was done to determine variability between germplasm and between different weeks using GenStat 15th edition. The scores obtained on disease severity from the screen house over the 6 weeks were converted into AUDPC values using the

Table 1. Maize germplasm used during the study.

No.	Germplasm	Description	No.	Germplasm	Description
1	KS23-6	Temperate line	20	39	Assorted tropical line
2	OHVRS-C1	Temperate line	21	5	Assorted tropical line
3	ks23-5	Temperate line	22	52	Assorted tropical line
4	OH28	Temperate line	23	3	Assorted tropical line
5	OH7B	Temperate line	24	35	Assorted tropical line
6	19	Assorted tropical line	25	CO80	Assorted tropical line
7	22	Assorted tropical line	26	50	Assorted tropical line
8	7	Assorted tropical line	27	60	Assorted tropical line
9	24	Assorted tropical line	28	DUMA	Kenyan hybrid
10	19	Assorted tropical line	29	17	Assorted tropical line
11	34	Assorted tropical line	30	30	Assorted tropical line
12	CO79	Assorted tropical line	31	114	Assorted tropical line
13	25	Assorted tropical line	32	119	Assorted tropical line
14	36	Assorted tropical line	33	18	Assorted tropical line
15	32	Assorted tropical line	34	112	Assorted tropical line
16	8	Assorted tropical line	35	12	Assorted tropical line
17	51	Assorted tropical line	36	9	Assorted tropical line
18	122	Assorted tropical line	37	14	Assorted tropical line
19	58	Assorted tropical line	38	16	Assorted tropical line

Sources: Author

Figure 1. Disease severity scale.
Source: Karanja et al. (2018).

formula:

$$AUDPC = \sum_{i=1} n-1[(t_{i+1} - t_i)(y_i + y_{i+1}) / 2]$$

where ‘‘t’’-time in days for each reading, ‘‘y’’-Disease score using the disease score (1-5), and ‘‘n’’-number of readings.

RESULTS

Response of maize germplasm to infection with MCMV

All the inoculated germplasm developed symptoms but disease severity differed significantly at $P < 0.01$ (Figure 2 and Table 2). Susceptible germplasm showed disease symptoms one week after the first inoculation. Leaf symptoms began as chlorotic strips running parallel to the veins that later joined to produce elongated chlorotic blotches (Figure 2B and C).

A t-test ($p<0.05$) confirmed there was no significant difference between season one and two for resistant germplasm and susceptible germplasm hence average of season one and season two was done for the resistance germplasm and susceptible germplasm. MCMV final severity scores of the 38 germplasm ranged from 2 to 4.3, the AUDPC ranged from 52.8 to 122.8 (Table 2). Five germplasm had a severity score of below 3. They

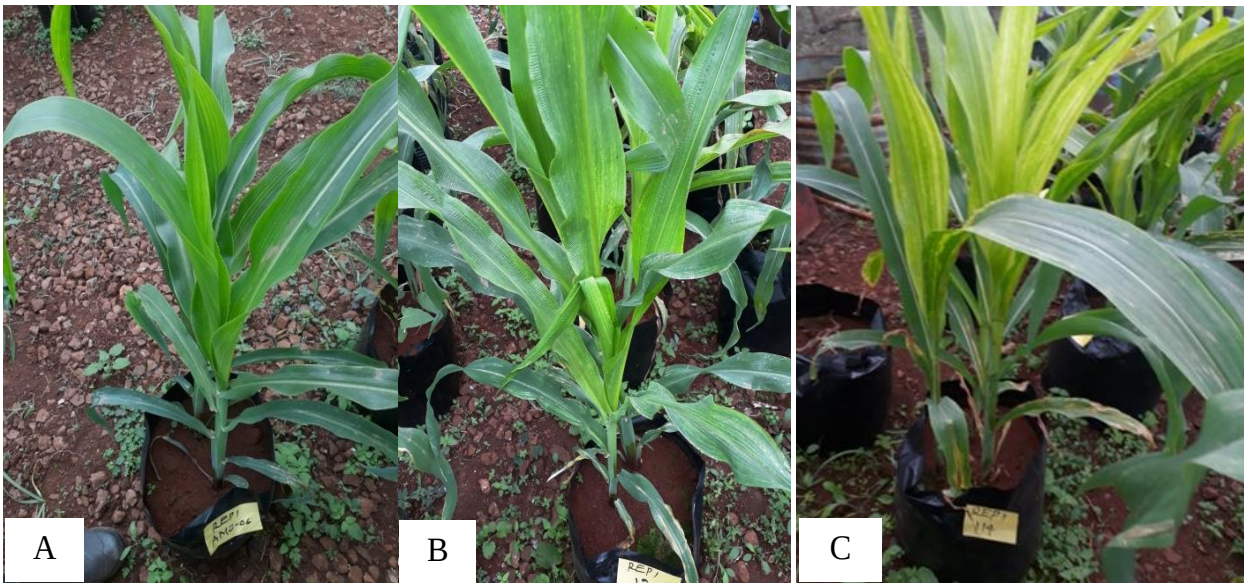

Figure 2. Variable *MCMV* disease severity observed during the trial. (A) Ks23-6 with low severity score of 1.5, (B) 19 with a medium severity of 3 and (C) 114 high severity score of 4.5.
Sources: Author

include germplasm Ks23-6 with a score of 2, germplasm 18, 9, 60, and Ks23-5 with a final score of 2.3 while 19 had a score of 3. Germplasm OH 28, 58, 14,122 and 58 had the highest scores of 4 or above (Table 2 and Figure 3). Germplasm 18 had the lowest AUDPC of 52.8 followed by Ks23-6 with 58.5, while 58 had the highest AUDPC of 122.8 (Table 2).

Response of maize germplasm to infection with SCMV

Infection was observed in all the plants inoculated with SCMV. Germplasm differed significantly at ($p< 0.001$) for resistance to SCMV.

Disease severity also differed over time at ($P<0.01$). The susceptible germplasm showed symptoms one week from the first inoculation. Final SCMV score ranged from 2.0 to 5 (Figure 4). Only two germplasm had a score of <2.5, that is, germplasm 50 with a score of 2.0 and germplasm 19 with 2.3. The germplasm 7, 22, and 48 had scores of 3 while germplasm 58 had the highest final score of 5.

Germplasm 19 and 7 showed low scores to both viruses. Germplasm 19 had a final severity score of 2.3 and AUDPC of 74.7 for SCMV trail and final severity score of 3.0 and AUDPC of 79.9 for MCMV trail while germplasm 7 had a final severity score of 3.0 and AUDPC of 79.9 for SCMV trail and final severity score of 2.7 and AUDPC of 66.7 for MCMV trail (Table 2, Figure 5).

DISCUSSION

Maize lethal necrosis disease is as a result of combined effect of MCMV and SCMV leading to yield losses of up to 100% (Gowda et al., 2015; Xia et al., 2016). Exposing plants to disease has been used to test and select germplasm for the presence of genes for resistance (Gowda et al., 2015). Previous work has reported that most elite inbred lines and commercial hybrids are susceptible to MCMV and MLND (Sitonik et al., 2019). This study partly agree with those previous report because among the studied germplasm, there was none that was immune to infection with either SCMV or MCMV. However there were significant differences in severity among different germplasm (Table 2). Earlier reports of work by Sitta et al. (2018), Karanja et al. (2018); Tembo et al. (2021) and Awata et al. (2021) where different germplasm were screened for MCMV, SCMV and MLND also reported development of symptoms on all screened germplasm but with different disease severity.

This study involved screening of 38 maize germplasm that are genetically diverse. Final severity/infection and AUDPC values were used as indicators of response of test germplasm to SCMV and MCMV (Tembo et al., 2021). There was significant difference between germplasm and between different scoring time/weeks at

Table 2. Weekly disease severity scores and the AUDPC of the germplasm studied.

No.	Genotype	SCMV Weekly Severity						AUDPC	No.	Genotype	MCMV Weekly Severity						AUDPC
		1	2	3	4	5	6				1	2	3	4	5	6	
1	50	1.0	1.0	1.0	1.0	1.5	2.0	42.0	1	KS23-6	1.2	1.3	1.5	1.8	1.8	2.0	58.5
2	19	1.0	1.3	2.5	2.8	2.3	2.3	74.7	2	18	1.2	1.2	1.2	1.6	1.9	2.3	52.8
3	7	1.2	1.7	2.3	2.5	2.8	3.0	79.9	3	ks23-5	1.1	1.1	1.7	2.1	2.2	2.3	61.1
4	22	1.0	1.2	1.3	1.7	2.5	3.0	60.7	4	9	1.3	1.3	1.5	1.5	1.8	2.3	59.8
5	48	1.0	1.2	1.5	2.7	3.0	3.0	72.3	5	60	1.5	1.5	1.7	1.7	1.7	2.3	59.8
6	34	1.0	1.2	2.2	2.3	2.8	3.0	73.5	6	19	1.2	1.5	2.2	2.7	3.0	3.0	60.3
7	24	1.0	1.2	2.7	3.0	3.2	3.0	84.0	7	22	1.3	1.5	1.5	1.8	2.2	3.5	65.9
8	25	1.0	1.5	2.8	3.0	3.2	3.2	88.1	8	7	1.7	1.5	1.7	1.8	2.3	2.7	66.5
9	5	1.0	1.3	2.5	2.5	3.3	3.3	81.4	9	24	1.3	1.5	1.5	1.8	2.3	3.3	66.5
10	15	1.0	1.2	2.0	2.5	3.0	3.3	75.8	10	19	1.5	1.5	1.7	1.8	2.3	3.0	67.1
11	16	1.0	1.5	2.5	2.8	3.0	3.3	84.0	11	34	1.5	1.5	1.5	2.0	2.5	2.8	67.7
12	OHVRS-C1	1.0	1.3	2.2	2.3	2.7	3.3	74.7	12	CO79	1.5	1.3	1.7	2.2	2.5	3.0	69.4
13	C080	1.3	1.7	2.5	3.0	3.5	3.3	91.0	13	25	1.5	1.7	1.7	2.2	2.5	3.0	71.8
14	3	1.0	1.3	2.7	2.7	3.2	3.5	84.6	14	36	1.5	1.5	1.5	2.2	2.8	3.2	72.3
15	36	1.2	1.7	2.8	3.2	3.3	3.5	93.3	15	32	1.3	1.3	1.5	2.0	3.3	3.5	72.6
16	60	1.0	1.3	2.0	2.5	2.8	3.5	76.4	16	8	1.3	1.5	1.5	2.2	3.0	3.3	73.5
17	119	1.0	1.5	2.5	2.8	3.3	3.5	86.9	17	51	1.3	1.5	1.8	2.2	2.7	3.5	74.1
18	KS23-6	1.0	1.0	1.8	2.2	2.7	3.5	69.4	18	39	1.5	1.5	1.7	2.2	3.0	3.2	74.7
19	OH7B	1.0	1.3	1.3	1.8	2.0	3.5	61.3	19	5	1.5	1.5	1.7	2.0	3.2	3.5	75.8
20	DUMA	1.0	1.2	2.7	3.2	3.5	3.5	89.3	20	52	1.3	1.5	1.5	2.0	3.5	3.5	76.4
21	18	1.0	1.5	2.5	2.8	3.3	3.7	87.5	21	3	1.5	1.3	1.5	2.2	3.5	3.7	77.6
22	30	1.0	1.2	1.8	2.0	3.2	3.7	73.5	22	35	1.2	1.3	1.3	2.5	3.5	3.7	77.6
23	35	1.0	1.5	2.3	3.2	3.2	3.7	87.5	23	CO80	1.5	1.5	1.5	2.5	3.2	3.3	77.6
24	39	1.0	1.7	2.5	2.8	3.2	3.7	87.5	24	50	1.3	1.3	1.8	2.7	3.7	3.7	84.0
25	9	1.0	1.2	1.5	2.8	3.7	3.8	81.1	25	OH7B	1.2	1.3	1.7	2.7	3.8	3.8	84.0
26	51	1.0	1.3	2.7	3.3	3.3	3.8	91.6	26	DUMA	1.2	1.2	1.7	3.5	3.5	3.5	85.4
27	CO79	1.0	1.3	3.0	3.3	3.5	3.8	95.1	27	17	1.0	1.5	1.5	1.5	3.0	3.0	66.5
28	8	1.0	1.3	2.8	3.5	4.0	4.0	99.2	28	30	1.2	1.3	1.8	3.2	3.5	4.0	86.9
29	12	1.0	1.5	2.3	3.3	3.5	4.0	91.0	29	114	1.3	1.5	2.0	2.5	3.8	3.8	86.9
30	14	1.0	1.8	3.0	3.8	3.5	4.0	101.5	30	119	1.5	1.5	1.5	3.2	3.7	3.8	87.5
31	52	1.0	1.3	2.2	3.0	3.7	4.0	88.7	31	OHVRS-C1	1.2	1.3	2.0	3.0	3.7	4.0	88.1
32	112	1.3	2.3	3.5	4.0	4.0	4.0	115.5	32	112	1.3	1.7	1.8	3.3	3.3	3.7	88.7

Table 2. Contd.

33	114	1.0	1.8	2.3	3.2	3.7	4.0	94.5	33	12	1.5	1.5	1.8	3.0	3.8	4.0	89.3
34	AMO-4	1.0	1.3	2.5	3.2	4.0	4.0	94.5	34	OH28	1.5	2.2	3.7	4.0	3.8	4.0	114.9
35	OH28/AMO-5	1.2	2.7	3.7	4.0	4.0	4.0	118.4	35	14	1.3	1.7	3.2	4.0	4.0	4.0	108.2
36	40	1.0	1.2	2.2	3.0	3.5	4.2	86.9	36	16	1.5	1.7	1.8	3.7	4.0	4.0	96.0
37	122	1.2	1.8	3.7	4.2	4.2	4.2	115.5	37	122	1.5	2.3	3.5	4.0	3.8	4.0	114.9
38	58	1.0	2.2	3.8	4.0	4.0	5.0	119.0	38	58	1.6	2.8	3.7	4.0	4.2	4.3	122.8

Sources: Author

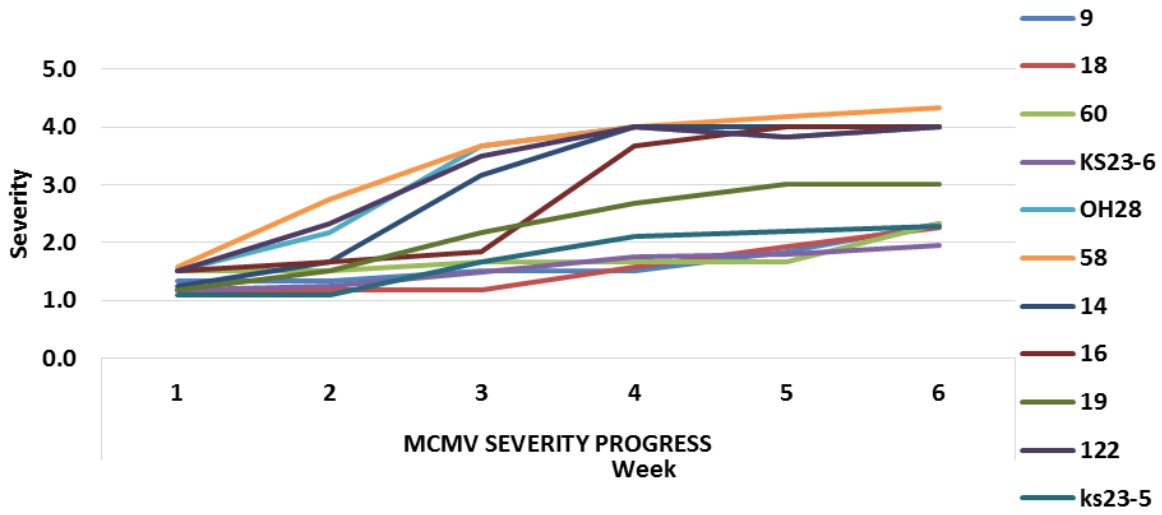

Figure 3. MCMV disease progress for the susceptible germplasm at the top and tolerant germplasm at the bottom derived from plotting disease severity scores over time (6 weeks).
Sources: Author

P<0.01; hence, the need for scoring at different time interval due to virus dynamics with time. High severity scores were recorded among the susceptible germplasm as the weeks progressed leading to high AUDPC. According to Karanja et al. (2020) and Sitta et al. (2017), germplasm can be classified as susceptible with a score 4 or above, tolerant with a score of 3 and resistance with a score of 2.

More than 80% of the studied germplasm were susceptible to SCMV and MCMV with scores of > 3.0, this puts emphasis on risk posed by MLND on

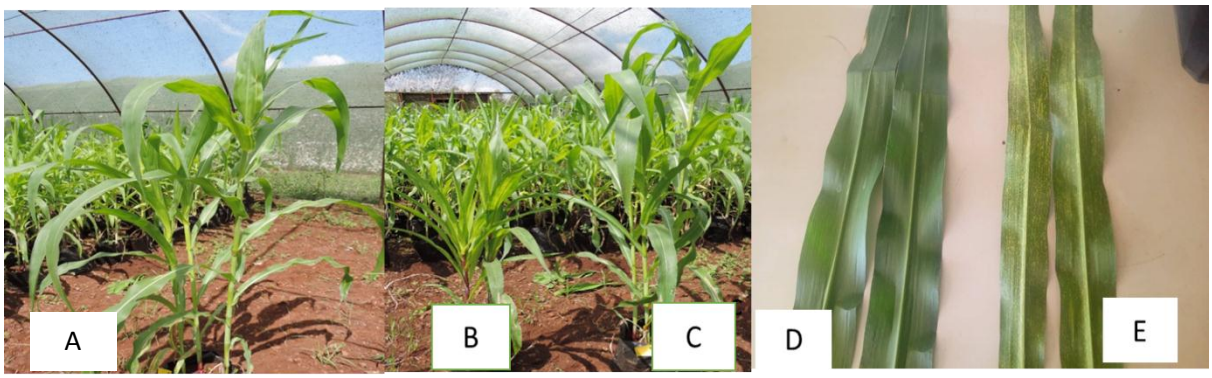

Figure 4. Variable *SCMV* disease severity observed during the trial. (A, C) Germplasm 50 with low severity score, (B) 58 high severity score, (D) leaves of germplasm 50 on the 6th week, (E) leaves of germplasm 58 at 6th week.
Sources: Author

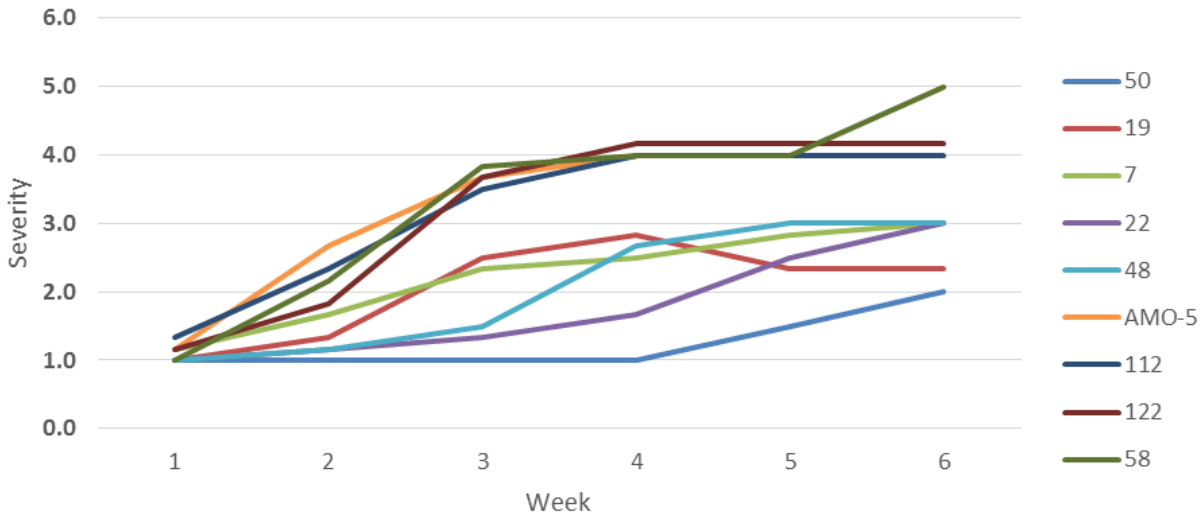

Figure 5. *SCMV* disease progress for the susceptible germplasm at the top and tolerant germplasm at the bottom derived from plotting disease severity scores over time (6 weeks).
Sources: Author

maize production and food security in the country. Germplasm 58, 28, 14, 122 and OH28 were the most susceptible to MCMV with a final score of ≥ 4 across the two seasons. Germplasm OH28, 112, 122 and 58 were the most susceptible to SCMV with severity score of ≥ 4 with the highest AUDPC of > 100. Three germplasms (OH28, 58, 122) were very susceptible to both viruses with the highest final score and AUDPC (Table 2), while germplasm 19 and 7 showed levels of resistance to both viruses. Paraschivu et al. (2013) reported a correspondence between germplasm AUDPC and susceptibility pointing that the most susceptible wheat germplasm had higher AUDPC values. This report is in agreements with the studies by Sitta et al. (2017) and Gowda et al. (2015) that reported high susceptibility of studied germplasm to MLND and causal agents.

Five germplasm showed tolerance to MCMV with a final score of < 3 across the six weeks and lowest AUDPC ranging from 58.5 to 61.1. Ks23-6 had the lowest score of 2 while germplasm 18,9,60, Ks23-5 had a score of 2.3 and germplasm 19 had a score 3.0. Evaluation of germplasm in response to SCMV suggest that germplasm 50 and19 are resistant with scores of below 2.5 while germplasm 7, 22 and 48 had a score of 3 meaning they are moderately tolerant. This study found that germplasm 7 and 19 may be having genes resistance

to both SCMV and MCMV with low severity scores and AUDPC in both trails (Table 2).

This study suggests that germplasm 18, 9, 60 and 19 may be carrying genes for MCMV resistant while germplasm 50 and 19 may be carrying genes resistant to SCMV. In addition, this study has confirmed that KS23-6 and KS23-5 are resistant to MCMV. KS23-6 and KS23-5 were identified as strong sources for MLND resistance and were developed by Kasetsart University in Thailand after crossing 26 inbred lines (Jones et al., 2018; Awata et al., 2021). Disease resistance is a mechanism developed by plants through evolution to survive attack by parasites. Quantitative trait loci (QTL) on chromosome six at 157 MB influences resistance to MCMV, as reported by Johns et al. (2018). It is inherited to the F2 population recessively. Two major genes Scmv1 and Scmv2 that confer resistance to Sugar cane mosaic virus have been mapped in various studies (Xia et al., 1999; Ingvardsen et al., 2010; Leng et al., 2017; Tao et al., 2013; Liu et al., 2009). More study on germplasm 18,9,60 needs to be carried out to confirm the presence of QTL that confers resistance to MCMV and for germplasm 50, 19, 22 and 48 to confirm presence of Scmv1 and Scmv2 responsible for SCMV resistance.

Conclusion

The results from this study show that the germplasm studied here are variable in response to MCMV and SCMV. The germplasm identified as tolerant in this research study could serve as potential donors to improve the adapted maize to combat MLND in the country. This will restore maize productivity and improve small scale farmer livelihood. Further studies should be done on the mode of inheritance of SCMV and MCMV resistance QTLs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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