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RESEARCH PAPER

Evaluating Early Generation Maize Lines for Resistance to Maize Streak Virus Under Artificial Infection Using Molecular Markers

Moïse Bizimana[1,3], Catherine Muui[1*], Lava Kumar[2] and Gedil Melaku[2]

[1]Kenyatta University, Department of Agricultural Science and Technology, Nairobi, Kenya
[2]International Institute of Tropical Agriculture (IITA), Oyo Road, Ibadan, Nigeria
[3]International Institute of Tropical Agriculture (IITA), Kabondo, Bujumbura, Burundi

*Corresponding author: muui.catherine@ku.ac.ke (**ORCID ID:** 0000-0001-6782-0626)

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ABSTRACT

Maize Streak Virus (MSV) disease is a major threat to cereal crops amongst smallholder farmers in sub-Saharan Africa. The purpose of this research was therefore, to identify lines with resistance to MSV using phenotypic and genotypic markers. To achieve this, early generation maize S1 lines were grown for phenotypic evaluation in the IITA greenhouse at Ibadan, Nigeria. Symptom severity was assessed visually using a 1–5 rating scale. Leaf tissues from infected maize were harvested and grinded for ELISA test. Besides, leaf tissues from healthy maize were harvested, dried for DNA extraction and shipped to the laboratory for SNP genotyping. The data on severity scores and recovery response was analysed using One-way ANOVA in SAS Software. The means were separated suing Tukey's post Hoc test and $p<0.05$ was considered statistically significant. The symptom severity scores differed significantly between the maize pedigree ($p = 0.0001$). The severity scores ranged from 4-5 within the first week of MSV inoculation with a mean incidence of 80%. Symptoms were recorded 3-5 days after the inoculations. Symptoms severity was significantly high on leaves found on the upper part compared to the leaves located on the lower plant parts. However, after two weeks, the symptoms severity declined with the lowest severity reported six (6) weeks after the inoculations. Twenty three maize lines had recovery response of >50% while 56 lines recorded response ranging 30 – 77.4% hence considered resistant. In contrast, 38 maize lines had a recovery response of < 30% and were considered susceptible. The highest AFV values were recorded in maize lines 35 (3.68), 49 (2.38), and 37 while the lowest values were recorded in maize lines 90, 44, and 75 respectively. Based on the four selected SNP markers, 56 maize lines were resistant to MSV, 16 lines were moderately resistant while 22 were susceptible. The maize lines which were resistant to MSV virus to be further screened for future use in breeding programs and subsequent distribution to farmers for production.

HIGHLIGHTS

- MSV virus is endemic to SSA causing significant economic impacts on increased maize yield loss, low income and increased prices of maize grain.
- Profiling the MSV resistant maize genotypes for utilization in breeding programs and subsequent distribution to farmers for production.
- Severity of infection was highest the first two weeks but reduced thereafter over time indicating their ability to resist the virus.
- MSV accumulated more on upper leaves than on lower leaves due to favourable conditions for the leaf hoppers vectors.
- Based on the four selected SNP markers, 56 maize lines were resistant to MSV, 16 lines were moderately resistant while 22 were susceptible.

Keywords: Maize, Maize streak Virus, phenotyping, genotyping, Molecular markers

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Food insecurity is a global problem that has raised concern in many countries (Offornedo *et al*. 2022) The global food demand is expected to double the current demand by 2050 due to increased human population and the socio-economic growth (Chivasa *et al*., 2021). In developing countries, meeting this demand requires that the current grain production be increased especially for maize, wheat and rice (Nair *et al*. 2015; Nasar *et al*. 2022; Sairam *et al*. 2025). Maize, which contributes 94% of the cereal production is one of the major crop considered to help in achieving food security particularly in Sub-Saharan Africa (SSA). Human population is projected to be approximately 10 billion by 2050 hence demand for food is expected to increase (Orebo *et al*. 2021). Production of crops particularly staple foods is constrained by climate change and an increase in pests and diseases. Consequently, it poses difficulties in achieving the desired global food security (Hickey *et al*. 2019).

In Africa, maize is considered a staple crop providing more than 50% calorie to over 27 million SSA population (Karavina, 2014; Ngeno, 2024). According to Cairns *et al*. (2021), maize occupies approximately 24% of the African cultivated farmlands. Besides, the annual yield is reported to be approximately two tons per hectare. Apart from being source of food, maize is a major source of income especially to the small holder farmers (Cheptoek *et al*. 2011; Ghosh *et al*. 2021; Manasa *et al*. 2021). The demand of this crop has been increasing and this has resulted to increase in area under maize production. However, the pattern of expansion-based production growth of maize crop production are not sustainable in many of the SSA countries (Afram *et al*. 2024; Sairam *et al*. 2024). Maize production is a function of various factors including climate, agronomy, genetics, policy and political stability (Shahane and Shivay, 2024). However, in SSA, the major constraining factors are climate change, diseases and pest invasions.

According to (Monjane *et al*. 2011), the production of maize is limited by both biotic and abiotic factors which not only compromise the quality and quantity of this crop but threatens food security. Climate change and its effects on crop pathogen vector appearance, behavior and distribution calls for a need to accelerate genetic improvement on the existing crops as a strategy of mitigating crop production constraints.

Maize streak virus (MSV) has been a threat to maize production in SSA. MSV is a virus in the genus *Masterevirus* and family *Geminiviridae*. In Africa, this virus is an important virus commonly transmitted through various species including *Cicadulina* leafhoppers (Afram *et al*. 2024). Besides, this virus is endemic in all maize varieties grown in the maize producing Sub Saharan Africa (SSA) countries. Currently, this virus has been reported in many African countries especially those in south of the Sahara. Since the virus was first reported in Africa in 1863, it moves transcontinentally at an average rate of 32.5 km/year (Monjane *et al*. 2011). In maize, MSV virus presents diverse symptoms including yellow streaks that run parallel to the leaf veins. Often, the symptoms of MSV infections are seen on emerging leaves after MSV infection while the older leaves may remain healthy. Besides, the susceptible maize cultivars develop streaking while infection at early stages result to severe stunting, interveinal necrosis and death of the infected plant.

According to Martin and Monjane, (2020), MSV infections results to significant maize loss with 100% loss reported in the susceptible maize lines. In SSA, most of the farmers are small holder farmers. According to Karavina, (2014), smallholder farmers report annual loss occasioned by MSV to be approximately $480 million. The effects of this invasion are exacerbated by high cost of inputs used in management of the MSV virus (Ngeno, 2024). Moreover, most of the small-holder farmers have limited knowledge and skill of managing the virus upon invasion of the maize farms hence increased damage and loss to this crop.

In Sub-Saharan Africa, maize streak virus (MSV) resistance necessitated the development of improved maize varieties with MSV resistance abilities (Welz *et al*. 1998; Shahane and Shivay, 2024). Resistance to MSV has previously been mapped to a main QTL (*Msv1*) on chromosome 1 and to several minor loci elsewhere in the genome (Nair *et al*. 2015). Similarly, genotyping using SNPs markers has been carried out using Kompetitive Allele Specific PCR, to find QTLs associated with MSV recovery resistance (Ladejobi *et al*., 2018). The genotyping technology has been successfully used in crops such as maize, rice, soybean, peanut, wheat among others. Following the validation of the KASP markers, genotyping has been successfully utilized in breeding programs particularly in allele mining, quantitative trait loci (GTL) mapping, QC analysis, and marker assisted recurrent selection (MARS).

To overcome the MSV constraints in maize production, IITA has introduced tropical maize lines with known resistance to generate backcrosses with elite lines as recipients of the favourable alleles. However, there is limited information on the genotyping information on each marker used in detecting the MSV associated allele. Besides, KASP essay are used in breeding due to its ability to improve the genotyping ability with low cost. Also, it is flexible, ease with data analysis and can be reproduced. Therefore, it can be used in profiling the MSV resistant maize genotypes and can be utilized in subsequent maize production. The current study was designed to screen early generation lines derived from backcrosses for resistance to MSV under artificial inoculation and using MSV-specific molecular markers.

MATERIALS AND METHODS

The study was carried out in IITA greenhouse in Ibadan, Nigeria, station. A total of 94 Maize lines were obtained from IITA research station Ibadan, Nigeria (Appendix 1). The maize lines were laid out in a completely randomized design (CRD) with three replicates. Eight seeds of each line and four seeds of the susceptible line (control: Pool-16) were sown in 1000kg pots. The pots were placed in cages in the greenhouse before the artificial virus inoculation.

Leafhopper (*Cicadilina triangular*) colonies that were used were obtained from the Virology and Molecular Diagnostics Unit of IITA in Ibadan station. The populations of leafhoppers were raised and fed with pearl millet (*Pennisetum typhoides*) seedlings and then kept in insect-proof cages. These insects are kept in cages and only removed when needed for artificial inoculation.

Planting of maize lines and management

The soil sampled from the field was sieved, sterilized and put in ninety-four 1000 g pots where six healthy seeds from each line were planted per pot. In addition, a control (GUSAU POOL 16) which is the most susceptible variety to MSV was planted for comparisons. Because an early treatment might kill or render leafhoppers ineffective, the pesticide (ACEPHATE 75 SP, was sprayed after inoculation to prevent the invasion of maize pests such as fall armyworm. Two to three hand weeding were done to keep the crop weed-free. Irrigation was done after planting and during the plant development.

Artificial inoculation of MSV by leafhopper

The maize inbred lines were phenotyped at the MSV phenotyping facility of the Virology & Molecular Diagnostics Unit of IITA-Ibadan using a standard procedure (Kumar *et al.,* 2009). The seeds of tests lines were planted in 12-inch diameter pots, with 6 seeds per pot along with susceptible control as inoculation check. For each line, three pots were used, and each pot was treated as one replicate. The populations of non-viruliferous leafhoppers which had been confirmed to be non-viruliferous using ELISA were supplied on clean pearl millet (*Pennisetum typhoides*) grown in the greenhouse. Adult leafhoppers were put to insect proof cages holding maize seedlings with severe MSV symptoms. The adult leafhoppers were allowed to stay in the insect proof cages for 48 hours to feed on and acquire the virus.

Determination of the response of maize lines to MSV under artificial infection

The viruliferous leafhoppers (leafhoppers which have not acquired the MSV virus) were introduced into cage containing young maize seedlings (5 to 7 days old), and exposed to MSV for a 48-h inoculation acquisition period. The plants were removed from their cages and treated with pesticide lambda-cyhalothrin (rate of 60 mL/20 l water) to eliminate vectors before being reinstalled to an insect-proof screen house. The plants were observed for appearance of first symptoms and monitored for symptom expression at weekly intervals and the symptoms were scored using a severity rating scale ranging from 1 to 5 (Kumar *et al.* 2009) for 6 weeks at regular intervals of a week. Measurements were done on plant height at 3, 6 and 10 weeks after planting. In harvested MSV-infected plants, the symptom severity score was recorded on each host plant.

Assessment of the incidence and viral symptom severity of leaves under MSV infection

Incidence was quantified as mean number of plants showing symptoms of MSV disease. The incidence of MSV disease was estimated using the formula below:

$$\text{Disease incidence } \% = \frac{\text{Number of plant attacked}}{\text{Total number of plants inspected}} \times 100\cdot$$

Therefore, MSV severity data were utilized to calculate the Area Under Disease Progress Curve (AUDPC), which quantifies the degree of MSV resistance or susceptibility.

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

Where: n = number of observation times, Y_i = disease severity at i^{th} observation time, and t_i = time in days at the i^{th} observation time (Simko and Piepho, 2012). The number of plants showing symptoms of MSV disease was recorded. The surface of the leaf infested by the MSV disease was estimated.

Determination of Relative Accumulation of MSV by Enzyme-Linked Immunosorbent Assay (ELISA)

At 6 week post inoculation (wpi), the leaves were harvested from two identical plants (with same symptoms score), from the fully expanded top leaf and the bottom leaf (which is still alive) in all the pots with the different inbred maize lines 1. The same sampling technique was used for the replicates. Two portions of tissue sample picked with a puncture for each line sample was grinded in its own mortar with 500 µl of coating buffer. The ELISA plate was covered, incubated for 1 hour at 37°C, washed three times with PBS -Tween by flooding for three minutes each time, and emptied before taping until it was dry. The wells were blocked with 200 µl per well of 3% Dried Skimmed milk in PBS – Tween 3g of milk in 100 µl of PBS.

The covered plates were incubated for 30 minutes at 37°C, and emptied before taping until it was dry. A100 µl per well of Polyclonal antibody (depending on the titter of the Antibody) was added in Conjugate buffer. To make a dilution of 1/5000 µl, 1g of leaf from healthy plant was grinded and put in 20 ml of conjugate buffer, plus 4µl of unpureed antibody, in order to remove all impurities. The covered plates were incubated for 1 hour at 37°C, washed three times with PBS -Tween by flooding for three minutes each time, and emptied before taping dry.

A100 µl per well of Goat Anti Rabbit Alkaline Phosphatase diluted in 1:15 was added in conjugate buffer (as recommended by manufacturer: 1 µl of enzyme in 15ml of conjugate buffer). The plates were incubated for 1 hour at 37°C, washed and emptied before taping until it was dry. A100 µl of P- nitrophenyl phosphate Substrate was added in Substrate Buffer, an absorbance was measured at 405 nm (A405 nm) using an ELISA plate reader (New Micro Read 1000, Global Diagnostics, Belgium) by reading after 1hour, 6 hours when the MSV antigen and the antibody reactions are completed. In each ELISA plate, virus positive control was MSV-infected Pool-16; uninfected maize was healthy control, and coating buffer while the control had no template.

The relative accumulation of the virus in the leaf extract based on the A405 nm values was estimated using an Absorbance Fold Value (AFV) (ratio) compared with the healthy control using the formula below as described by Sime *et al.* (2021):

$$AFV = \frac{\text{A405nm of Test line's leaf}}{2\text{X}\left(\text{A405nm of healthy control's leaf}\right)}$$

Screening of maize S1 lines using MSV-specific KASP SNP markers

Inbred maize seeds were planted in pots at a greenhouse at IITA station, Ibadan. During planting, eight seeds for the test lines and the control were planted in each pot.

Ten young leaves from non-inoculated plants were harvested from each maize line and bulked in jute bags two weeks after planting. The bulked leaf samples were lyophilized and then turned into powder by shaking at 1500 strokes per min for 1.5 min using a high-speed automated tissue homogenizer, Geno/Grinder 2010 (SPEX Sample Prep) at the IITA Bioscience Center in Ibadan, Nigeria. The CTAB DNA extraction protocol was used to extract genomic DNA from ground leaf tissues (Aboul-Maaty and Oraby, 2019).

KASP genotyping

The KASP genotyping components include KASP-

TF Master Mix, KASP Assay Mix designed for a specific target, and template DNA. The extracted DNA, diluted to a working concentration, was used as the template for KASP genotyping. MSV-specific KASP markers was used to screen and identify the S1 maize lines with desirable alleles of the SNP markers linked to QTL associated with MSV resistance in maize. According to the KASP genotyping guide, the KASP reaction was conducted in a 96-well plate with a total reaction volume of 10 µl consisting of 5 µl template DNA and 5 µl of the prepared genotyping mix (2× KASP master mix and primer mix) (Cruz *et al*. 2017). To run the KASP amplification reaction, a Light Cycler 480 II RT-PCR System was used (Roche Life Sciences, Germany). One cycle of KASP Taq activation at 94°C for 15 minutes was set, proceeded by 36 denaturation cycles at 94°C for 20 seconds, annealing and stretching at 60°C for 1 minute. The fluorescence signal's endpoint was detected for 1 minute at 30°C.

The genotypic data were collected, as genotype calls, via the KASP genotyping platform following the screening of the S1 maize lines to identify lines carrying the favourable alleles associated with MSV resistance. The genotype calls were recorded as homozygous for the beneficial gene, homozygous for the undesirable gene, or heterozygous for both genes.

Data analysis

Percentage incidence of MSV infection was established by enumeration of infected maize plants out of the total maize plants planted in each pot and expressed as a percentage. The data obtained was normalized for statistical analysis using percentage incidences on each maize line. Using Stata version 17.0, statistical software, analysis of variance (One-way ANOVA) to show differences in % incidence in maize lines was carried out at 95 % confidence interval. Where there were differences, the means were separated using Tukey`s HSD.

The data for Symptom Severity Score (SSS: According to the concentration of streaks on the leaf of each host plant), data was also normalized and subjected to the Analysis of variance (ANOVA) tool available in Stata software version 17. Tukey's post Hoc test was used to separate the means. Kluster Caller analysis software was used to analyse the genotype calls. The amplified products' genotypes were presented in a Cluster plot and categorized as homozygous for gene 1 (allele reported by FAM fluorophore), homozygous for gene 2 (gene reported by HEX fluorophore), heterozygous (genes reported by FAM and HEX fluorophores). The SNP viewer software was used to view and download the cluster plot image.

RESULTS

Symptom severity of leaves after MSV infection

In this study, 94 genotype maize lines and the controls had varying level of resistance to the MSV infection under greenhouse conditions. The severity scores differed significantly between the maize pedigree ($p = 0.0001$) (Table 1). The inoculations with viruliferous leafhoppers resulted to transmission of the MSV in the maize test plants and the controls. The severity scores ranged from 4-5 within the first week of MSV inoculation. The symptoms of the MSV infections in maize plants included chlorotic streaks. However, the severe symptoms of the MSV infection occurred in leaves which emerged after the inoculation. The mean incidence was 80 % after one week of inoculation (Plate 1).

Plants inoculated with the MSV virus developed the MSV symptoms 3-5 days after the inoculations (Table 1). In each plant, the symptoms severity of the leaves on the upper part of the plant differed from those on the lower part of the plant with the severity being significantly high on leaves found on the upper plant compared to the leaves located on the lower plant parts. However, the symptom severity significantly increased to maximum 2 weeks after inoculation. After the two weeks, the symptoms severity declined with the lowest severity reported six (6) weeks after the inoculations (Plate 1).

Maize pedigree 91, 75, and 8 had significantly lower severity scores six weeks after inoculation (Table 1). The severity scores differed significantly between the maize lines. These scores were significantly high within the first two weeks. This findings agrees with Sime *et al*. (2021), who indicated that the severity scores of MSV virus in maize line was high within the first two weeks and reduced with time. In the first two weeks after infection, MSV virus rapidly replicates and spreads throughout the plant tissues. In this stage, the MSV virus infection

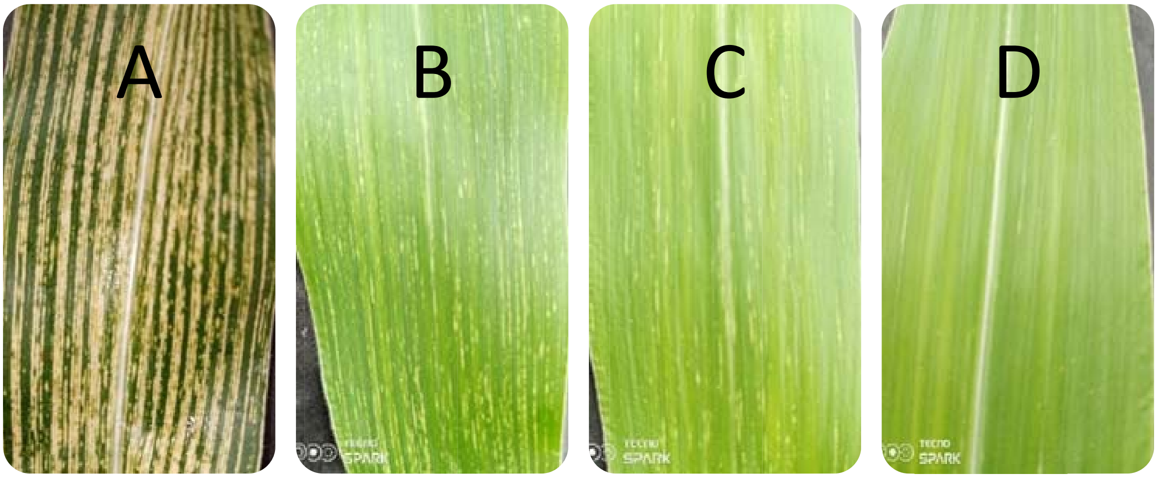


Plate 1: Reduction in symptom severity of a maize line after inoculation, with recovery resistance MSV infection. **Left to right:** the emerged leaf showing severe symptoms (*A*) and a gradual reduction in severity in the next leaves (B-D)

is characterized with aggressive viral activity which causes significant symptoms including chlorosis, stunted growth and leaf streaking (Kaur *et al.* 2022). These symptoms contribute to the high severity scores within the first week of inoculation. As the infection progresses from week 3-6, the severity reduced and this can be attributed to the host maize plant ability to activate its defense mechanism including systemic acquired resistance (SAR) and physiological changes aimed to combat the virus and its effects. Therefore, between 3- 6 weeks of infection, the maize plant defenses response is more effective leading to reduced visible symptoms and consequently lower severity scores (Sime *et al.* 2021).

The viral load in the host plant stabilizes over time thus reduced severity of symptoms. Besides, as the disease progresses, the plant undergo adaptive changes which allow it to better tolerate the virus presence hence reduced severity. Moreover, environmental conditions such as temperature, light and humidity influence the MSV severity symptoms (Jeger, 2023). During the initial infections, the environmental conditions may favor rapid MSV virus replication. However, as the host plant adjusts to the infection and environmental conditions potentially change and the symptoms diminish as described by Sime *et al.* (2021).

The differences in severity scores of MSV virus on the different maize lines may be attributed to differences in genetic resistance and susceptibility to this virus. The MSV resistant MSV lines have resistant genes and this limits replication of the virus within the cells (Jiang and Zhou, 2023). In some cases, the resistance ability may limit the spread of the virus from cell to cell or through the plant is restricted (Jeger, 2023). Besides, some resistant plants may exhibit hypersensitive response where the infected cells undergo programmed cell death to limit the spread of the infected MSV virus (Sharma *et al.* 2021). In contrast, the susceptible maize lines may not have the resistant mechanism hence show severe symptoms upon infection by the MSV virus (Monjane *et al.* 2020b). The susceptibility of some maize lines can be attributed to the specific alleles on the maize genome which confer susceptibility to the virus including MSV virus (Tatineni and Hein, 2023).

Area Under Disease Progress Curve (AUDPC) and recovery response of inbred maize lines after artificial MSV inoculation

In this study, the percentage recovery in the tested maize lines ranged from -11.3 to 77.4 %. Out of the tested 94 maize lines, only 23 maize lines had recovery response of >50. Besides, 71 of the lines had <50% recovery. A total of 56 maize lines had recovery response ranging from 30% -77.4% hence they were considered as resistant R (a). The highest % recovery was reported on maize plant on the pedigree (TZISTR1233/KS23-6/TZISTR1233)-54 with % recovery of 77.4%, (TZISTR1219/KS23-3/TZISTR1219)-91 (75.6%), (TZISTR1233/KS23-6/TZISTR1233)-23 (75.4) and (TZISTR1233/KS23-6/TZISTR1233)-37 (75.0%) (Table 2).

In contrast, 38 maize lines had a recovery response of < 30% and were considered susceptible (S).

Table 1: Symptoms severity scores after artificial inoculations with the MSV Virus

Pedigree	**SSS Week 1**	**SSS Week 2**	**SSS Week 3**	**SSS Week 4**	**SSS Week 5**	**SSS Week 6**
1	3.76±0.13ab	4.19±0.19abc	5.00±0.00a	4.77±0.10ab	4.38±0.13abcdefg	4.31±0.19abcdefgh
2	4.81±0.06a	4.75±0.13ab	4.94±0.06a	4.81±0.06ab	4.69±0.06abc	4.81±0.06abc
3	4.44±0.06a	4.56±0.06ab	4.56±0.06ab	3.50±0.13abcdefghijk	2.75±0.25hgijklmnopq	2.25±0.00opqrstuwxyzab
4	4.21±0.21ab	3.80±0.20abc	3.80±0.20abc	3.21±0.21bcdefghijk	2.70±0.30hgijklmnopq	2.56±0.16mnopqrstuwxyz
5	4.10±0.10ab	4.10±0.10abc	4.24±0.04ab	4.66±0.06abc	3.98±0.18abcdefhgij	3.49±0.08bcdefghijklmnop
6	4.30±0.30ab	4.40±0.40ab	4.57±0.23ab	4.83±0.17ab	4.28±0.12abcdefgh	3.63±0.03abcdefghijklmno
7	4.24±0.09ab	4.00±0.00abc	4.00±0.00abc	4.00±0.00abcdefgh	3.30±0.02bcdefhgijklmno	3.14±0.14fghijklmnopq
8	4.06±0.06ab	3.33±0.67abc	3.50±0.50abc	3.44±0.44abcdefghijk	2.67±0.33ijklmnopq	2.48±0.14nopqrstuwxyzab
9	4.08±0.08ab	3.92±0.08abc	3.91±0.08abc	4.33±0.67abcde	3.50±0.50abcdefhgijk	2.83±0.17ijklmnopqrst
10	4.00±0.00ab	4.00±0.00abc	4.00±0.00abc	2.92±0.08efghijk	2.50±0.17ijklmnopqr	1.67±0.00rstuwxyzab
11	4.10±0.10ab	4.40±0.00ab	4.20±0.20ab	3.70±0.10abcdefghij	3.20±0.20cdefhgijklmnop	2.50±0.10nopqrstuwxyzab
12	4.36±0.07ab	4.21±0.21abc	4.07±0.07abc	3.86±0.00abcdefgh	3.43±0.00abcdefhgijkl	3.07±0.07fghijklmnopqr
13	4.28±0.48ab	4.32±0.08ab	4.20±0.20ab	3.78±0.03abcdefghi	3.43±0.18abcdefhgijkl	3.20±0.20efghijklmnopq
14	4.76±0.04a	4.83±0.03ab	4.83±0.03a	4.00±0.00abcdefgh	3.37±0.23abcdefhgijklm	3.20±0.20efghijklmnopq
15	2.50±2.50ab	2.44±2.44abc	2.38±2.38c	2.00±2.00k	1.75±1.75klmnopqr	1.44±1.44tuwxyzab
16	5.00±0.00a	5.00±0.00a	5.00±0.00a	4.54±0.04abcd	3.80±0.05abcdefhgijk	3.13±0.01fghijklmnopq
17	4.28±0.15ab	4.47±0.16ab	4.21±0.21ab	3.88±0.13abcdefgh	2.96±0.33efhgijklmnopq	2.60±0.03mnopqrstuwx
18	5.00±0.00a	5.00±0.00a	5.00±0.00a	4.83±0.17ab	3.83±0.17abcdefhgij	3.50±0.17bcdefghijklmnop
19	4.58±0.08a	4.71±0.04ab	4.44±0.06ab	4.00±0.00abcdefgh	3.35±0.02abcdefhgijklmn	3.27±0.10defghijklmnop
20	5.00±0.00a	4.63±0.13ab	4.56±0.06ab	3.77±0.10abcdefghhi	3.00±0.00defhgijklmnopq	2.56±0.06mnopqrstuwxyz
21	5.00±0.00a	4.94±0.06a	4.85±0.02a	4.00±0.00abcdefgh	3.31±0.19bcdefhgijklmno	2.92±0.08mnopqrstuwxy
22	5.00±0.00a	5.00±0.00a	5.00±0.00a	4.02±0.35abcdefgh	3.52±0.85abcdefhgijk	1.67±0.58ghijklmnopqrs
23	4.00±0.00ab	3.72±0.15abc	3.32±0.18abc	2.06±0.06jk	2.13±0.01klmnopqr	2.72±0.04rstuwxyzab
24	4.00±0.00ab	4.00±0.00abc	4.38±0.24abc	4.12±0.23abcdefgh	3.48±0.23abcdefhgijk	2.72±0.15lmnopqrstuv
25	4.44±0.44 ab	4.31±0.31ab	3.88±0.13ab	3.81±0.19abcdefgh	3.81±0.19abcdefhgijk	2.69±0.06mnopqrstuw
26	3.87±0.01ab	3.09±0.34abc	2.88±0.13abc	2.14±0.14ijk	1.66±0.08nopqr	1.26±0.12wxyzab
27	4.20±0.20ab	4.20±0.20abc	4.47±0.33ab	4.76±0.04ab	4.76±0.04abc	4.41±0.01abcdef
28	4.40±0.00ab	3.63±0.00abc	4.06±0.069abc	3.88±0.00abcdefgh	3.69±0.06abcdefhgijk	3.44±0.06bcdefghijklmnop
29	5.00±0.00a	5.00±0.00abc	5.00±0.00a	5.00±0.00a	4.88±0.13a	4.63±0.13abcde
30	3.92±0.08ab	4.00±0.00abc	4.94±0.06a	4.88±0.13ab	4.71±0.04abc	4.33±0.33abcdeefg
31	4.00±0.00ab	3.94±0.06abc	3.71±0.04abc	3.58±0.08abcdefghijk	3.58±0.08abcdefhgijk	3.46±0.21bcdefghijklmnop
32	3.93±0.07ab	3.93±0.07abc	3.86±0.14abc	3.86±0.14abcdefgh	3.52±0.19abcdefhgijk	3.46±0.12bcdefghijklmnop
33	4.30±0.30a	3.85±0.05abc	3.55±0.25abc	3.00±0.00cdefghijk	1.55±0.05pqr	1.05±0.05a′b′
34	5.00±0.00a	5.00±0.00a	5.00±0.00a	4.3±0.00abcde	3.90±0.00abcdefhgij	3.25±0.15defghijklmnop
35	4.00±0.00ab	3.69±0.06abc	3.63±0.00abc	3.56±0.06abcdefghijk	3.69±0.06abcdefhgijk	3.69±0.06abcdefghijklmno
36	4.00±0.00ab	4.00±0.00abc	3.83±0.17abc	4.00±0.00abcdefgh	3.80±0.20abcdefhgijk	2.87±0.46hijklmnopqrst
37	3.80±0.20ab	3.68±0.08abc	3.68±0.07abc	3.24±0.36abcdefghijk	2.91±0.29fhgijklmnopq	1.65±0.15rstuwxyzab
38	4.47±0.13a	4.82±0.02ab	4.82±0.02a	4.45±0.05abcd	4.10±0.10abcdefhgij	3.28±0.12defghijklmnop
39	3.94±0.06ab	3.79±0.04abc	3.79±0.04abc	3.42±0.08abcdefghijk	2.50±0.00ijklmnopqr	1.60±0.27stuwxyzab
40	3.52±0.33ab	3.83±0.03abc	3.83±0.03abc	3.83±0.03abcdefgh	3.19±0.39defhgijklmnopq	1.61±0.18stuwxyzab
41	5.00±0.00a	1.42±0.08ab	4.92±0.08a	4.00±0.00abcdefgh	4.00±0.17abcdefhgij	3.00±0.17fghijklmnopqrs
42	4.92±0.08a	4.79±0.04ab	4.21±0.04ab	4.45±0.05abcd	3.88±0.13abcdefhgij	3.21±0.04efghijklmnop
43	4.61±0.11a	4.38±0.05ab	3.75±0.25abc	4.70±0.13ab	3.46±0.04abcdefhgijk	2.09±0.24pqrstuwxyzab
44	4.63±0.20a	4.15±0.01abc	4.61±0.11ab	4.25±0.25abcde	4.69±0.02abc	2.90±0.24ghijklmnopqrs
45	4.61±0.11a	4.45±0.12ab	4.77±0.06a	3.75±0.25abcdefghi	4.04±0.46abcdefhgij	3.19±0.48efghijklmnopq
46	4.44±0.16a	4.17±0.03abc	4.17±0.03ab	4.00±0.00abcdefgh	3.90±0.10abcdefhgij	2.17±0.03pqrstuwxyzab

47	4.14±0.14ab	4.14±0.14abc	4.00±0.00abc	3.67±0.33abcdefghij	3.86±0.14abcdefhgij	2.25±0.25opqrstuwxyzab
48	5.00±0.00ab	5.00±0.00a	4.25±0.08a	5.00±0.00a	4.00±0.00abcdefhgij	3.25±0.08defghijklmnop
49	4.50±0.50a	4.51±0.20ab	4.43±0.43ab	4.50±0.50abcd	3.90±0.23abcdefhgij	3.38±0.05cdefghijklmnop
50	4.48±0.23a	4.28±0.15ab	4.13±0.01abc	5.00±0.00a	3.28±0.15bcdefhgijklmno	1.21±0.08xyzab
51	4.88±0.13a	5.00±0.00a	5.00±0.00a	5.00±0.00a	4.86±0.14a	4.85±0.14ab
52	4.93±0.07 ab	5.00±0.00a	5.00±0.00a	4.53±0.04abcd	4.39±0.11abcdef	4.67±0.04abcd
53	5.00±0.00a	5.00±0.00a	5.00±0.00a	5.00±0.00a	5.00±0.00a	5.00±0.00a
54	5.00±0.00a	5.00±0.00a	5.00±0.00a	4.20±0.20abcdefg	3.90±0.10abcdefhgij	3.65±0.15abcdefghijklmno
55	4.79±0.07a	4.64±0.07ab	4.28±0.14ab	3.93±0.21abcdefgh	3.36±0.07abcdefhgijklmn	3.43±0.00bcdefghijklmnop
56	5.00±0.00a	5.00±0.00a	5.00±0.00a	4.54±0.04abcd	4.57±0.00abcd	3.82±0.32abcdefghijklmn
57	4.36±0.38a	4.83±0.17ab	4.83±0.17a	3.88±0.13abcdefgh	4.00±0.00abcdefhgij	3.88±0.13abcdefghijklmn
58	4.63±0.38a	5.00±0.00a	5.00±0.00a	4.88±0.13ab	4.75±0.25abc	4.63±0.38abcde
59	5.00±0.00a	4.75±0.25ab	4.75±0.25a	4.00±0.00abcdefgh	3.75±0.25abcdefhgijk	2.75±0.25klmnopqrstu
60	5.00±0.00a	4.50±0.50ab	3.83±0.50abc	3.67±0.33abcdefghij	3.37±0.33abcdefhgijk	2.33±0.33opqrstuwxyzab
61	4.92±0.08a	4.69±0.02ab	4.77±0.06a	4.70±0.13ab	4.11±0.39abcdefhgi	2.51±0.09nopqrstuwxyza
62	5.00±0.00a	4.83±0.17ab	4.83±0.17a	4.25±0.25abcde	3.75±0.25abcdefhgijk	2.75±0.25klmnopqrstu
63	4.75±0.25a	4.75±0.25ab	4.25±0.25ab	3.75±0.25abcdefghi	3.25±0.25bcdefhgijklmnop	1.75±0.25qrstuwxyzab
64	5.00±0.00a	4.90±0.10ab	4.47±0.13ab	4.20±0.20abcdefg	4.03±0.37abcdefhgij	3.50±0.50bcdefghijklmnop
65	4.90±0.10 ab	5.00±0.00a	5.00±0.00a	4.32±0.07abcde	4.10±0.10abcdefhgij	2.65±0.15mnopqrstuwx
66	4.66±0.09a	5.00±0.00a	5.00±0.00a	4.73±0.02ab	4.61±0.10abcd	3.80±0.05abcdefghijklmn
67	4.88±0.13a	5.00±0.00a	5.00±0.00a	4.88±0.13ab	4.88±0.13a	4.25±0.25abcdefghi
68	4.75±0.25a	5.00±0.00a	5.00±0.00a	5.00±0.00a	4.75±0.25abc	4.62±0.13abcde
69	4.88±0.13a	5.00±0.00a	5.00±0.00a	4.42±0.08abcd	4.17±0.17abcdefhgi	4.17±0.17abcdefghijkl
70	4.87±0.01a	4.94±0.06a	4.94±0.06a	4.60±0.03abc	4.07±0.07abcdefhgij	2.79±0.08jklmnopqrst
71	4.67±0.00a	5.00±0.00a	4.67±0.00a	4.33±0.00abcde	4.17±0.17abcdfgh	3.67±0.00abcdefghijklmno
72	5.00±0.00a	5.00±0.00a	5.00±0.00a	5.00±0.00a	5.00±0.00a	4.20±0.20abcdefghijk
73	4.18±0.02ab	4.63±0.03ab	4.53±0.13ab	4.53±0.13abcd	4.00±0.00abcdefhgij	2.81±0.02ijklmnopqrst
74	4.71±0.04a	4.67±0.17ab	4.67±0.17a	4.13±0.13abcdefgh	4.00±0.00abcdefhgij	3.63±0.13abcdefghijklmno
75	4.68±0.18a	4.88±0.13ab	4.36±0.36ab	3.88±0.13abcdefgh	3.80±0.05abcdefhgijk	2.86±0.14ijklmnopqrst
76	4.83±0.17a	5.00±0.00a	5.00±0.00a	5.00±0.00a	5.00±0.00a	4.50±0.04abcdefghij
77	5.00±0.00a	5.00±0.00a	5.00±0.00a	5.00±0.00a	5.00±0.00a	4.17±0.17abcdefghijkl
78	5.00±0.00a	5.00±0.00a	5.00±0.00a	5.00±0.00a	5.00±0.00a	4.00±0.00abcdefghijklm
79	4.38±0.00a	4.81±0.06ab	4.56±0.06ab	4.16±0.19abcdefg	4.06±0.18abcdefhgij	2.94±0.06ghijklmnopqrs
80	4.44±0.06a	4.38±0.13ab	4.06±0.06abc	2.75±0.25efghijk	2.44±0.06jklmnopqr	1.55±0.05stuwxyza′b′
81	3.94±0.06ab	3.75±0.13abc	3.63±0.13abc	2.50±0.00hijk	1.19±0.06r	1.06±0.06a′b′
82	5.00±0.00a	4.93±0.07a	4.93±0.07a	4.54±0.04abcd	3.00±0.00defhgijklmnopq	1.67±0.04rstuwxyzab
83	4.64±0.07a	3.71±0.14abc	3.57±0.00abc	3.36±0.07abcdefghijk	1.38±0.04pqr	1.14±0.00yzab
84	4.90±0.10a	4.28±0.12ab	3.83±0.17abc	3.57±0.23abcdefghijk	1.57±0.23opqr	1.28±0.12wxyzab
85	3.73±0.02ab	3.46±0.04abc	3.27±0.02abc	2.55±0.30fghijk	1.68±0.17nopqr	1.28±0.02wxyzab
86	5.00±0.00a	4.50±0.25ab	4.38±0.13ab	3.50±0.13abcdefghijk	1.68±0.06lmnopqr	1.25±0.13wxyzab
87	4.71±0.04a	4.10±0.23abc	3.48±0.14abc	2.58±0.08fghijk	1.13±0.13r	1.06±0.06a′b′
88	3.94±0.06ab	3.79±0.04abc	3.56±0.06abc	2.54±0.21ghijk	1.20±0.04qr	1.06±0.06a′b′
89	4.25±0.00a	3.88±0.13abc	3.50±0.00abc	2.50±0.13hijk	1.38±0.13qr	1.13±0.13zab
90	4.40±0.03a	3.94±0.06abc	3.73±0.02abc	3.27±0.10bcdefghijk	1.72±0.15lmnopqr	1.33±0.04uwxyzab
91	4.45±0.05a	4.90±0.10ab	4.72±0.12a	4.82±0.02ab	4.08±0.08abcdefhgij	3.28±0.12defghijklmnop
92	5.00±0.00a	5.00±0.00a	5.00±0.00a	5.00±0.00a	4.35±0.22abcdefg	3.80±0.05abcdefghijklmn
93	4.94±0.06a	4.25±0.00abc	4.38±0.00ab	4.69±0.06ab	4.00±0.25abcdefhgij	3.38±0.25cdefghijklmnop
94	4.83±0.17a	4.75±0.25ab	4.63±0.13ab	4.21±0.04abcdef	3.63±0.13abcdefhgijk	2.92±0.08ghijklmnopqrs

Means with the same letters are not statistically significant at p<0.05 based on Tukey's post Hoc Test. Key: SSS-symptoms severity scores.

Majority of the maize lines classified as susceptible had negative % recovery value. This means that the inoculated maize plant had low severity scores one week after inoculation (1wpi) and became severely infected until 6 wpi (week post inoculation). These included maize lines in pedigree (TZISTR1211/KS23-3/TZISTR1211)-104 with % recovery of -11.3%, (TZISTR1219/KS23-3/TZISTR1219)-85 (-10.6 %) and (TZISTR1219/KS23-3/TZISTR1219)-79 (-5.1%) (Table 3).

Relative MSV Titer on Inbred Maize Lines Leaves

Based on the Absorbance fold value (AFV) of top leaves (least symptoms), 60 lines showed resistance to MSV, whereas 34 maize lines were susceptible. At the bottom of the leaves only 17 lines were resistant (R) while 77 lines were categorized as susceptible (S) based on the AFV values. The highest AFV values were recorded in maize lines 35 (3.68), 49 (2.38), and

Table 2: Area Under Disease Progress Curve (AUDPC) of inbred maize lines after artificial MSV inoculation

Entry	AUDPC	Entry	AUDPC	Entry	AUDPC
1	156.99±0.07abcdefgh	36	133.47±1.87abcdefghijklmn	71	156.33±1.17abcdefgh
2	168.00±0.00abcde	37	113.47±3.31fghijklmno	72	172.20±0.70ab
3	131.03±1.53abcdefghijklmn	38	154.41±0.99abcdefghi	73	148.40±2.10abcdefghijk
4	118.30±7.70cdefghijklmno	39	113.90±0.73fghijklmno	74	151.37±0.88abcdefghij
5	145.43±1.23abcdefghijklm	40	120.70±3.80bcdefghijklmno	75	144.75±4.75abcdefghijklm
6	154.35±7.35abcdefghi	41	149.33±1.75abcdefghijk	76	171.65±0.73abc
7	133.00±0.00abcdefghijklmn	42	149.71±0.09abcdefghijk	77	172.08±0.58ab
8	113.88±14.29fijklmno	43	137.54±2.29abcdefghijklmn	78	171.50±0.00abc
9	133.88±9.63abcdefghijklmn	44	150.29±2.71abcdefghijk	79	148.97±1.53abcdefghijk
10	113.75±1.75fijklmno	45	146.37±2.38abcdefghijklm	80	116.33±1.27defghijklmno
11	131.60±0.70abcdefghijklmn	46	136.85±0.35abcdefghijklmn	81	94.94±1.31lmno
12	135.00±2.00abcdefghijklmn	47	132.04±1.96abcdefghijklmn	82	145.09±0.59abcdefghijklm
13	136.24±2.36abcdefghijklmn	48	156.63±0.88abcdefgh	83	104.42±1.08hijklmno
14	147.05±2.05abcdefghijklm	49	148.97±11.53abcdefghijk	84	114.39±5.31efghijklmno
15	73.72±73.72o	50	136.72±3.28abcdefghijklmn	85	94.94±3.25mno
16	156.84±0.66abcdefgh	51	173.06±1.06ab	86	120.31±2.63bcdefghijklmno
17	132.25±6.25abcdefghijklmn	52	166.09±0.59abcdef	87	99.24±0.07jklmno
18	160.42±2.92abcdefg	53	175.00±0.00a	88	95.23±1.90lmno
19	142.99±0.07abcdefghijklm	54	156.98±2.63abcdefgh	89	97.56±1.31klmno
20	138.18±2.26abcdefghijklmn	55	142.25±1.75abcdefghijklm	90	108.71±1.54ghijklmno
21	146.27±1.90abcdefghijklm	56	164.63±1.38abcdef	91	156.68±1.98abcdefgh
22	150.50±10.50abcdefghijk	57	151.67±2.33abcdefghij	92	166.25±1.75abcdef
23	98.53±2.53jklmno	58	169.75±5.25abcd	93	150.28±3.28abcdefghijk
24	135.41±2.41abcdefghijklmn	59	147.87±6.13abcdefghijklm	94	147.58±4.67abcdefghijklm
25	135.63±0.44abcdefghijklmn	60	135.33±12.83abcdefghijklmn		<.0001
26	78.31±3.19no	61	153.93±3.93abcdefghi		
27	157.45±4.95abcdefgh	62	150.79±3.21abcdefghijk		
28	132.78±0.66abcdefghijklmn	63	134.75±8.75abcdefghijklmn		
29	172.81±1.31ab	64	152.95±5.95abcdefghi		
30	158.52±1.90abcdefg	65	155.40±1.40abcdefghi		
31	129.79±2.04abcdefghijklmn	66	165.00±0.50abcdef		
32	132.00±1.00abcdefghijklmn	67	170.19±3.06abc		
33	102.38±3.68ijklmno	68	171.06±3.06abc		
34	156.28±0.53abcdefgh	69	161.73±1.60abcdefg		
35	128.84±0.66abcdefghijklmn	70	156.63±0.88abcdefgh		

Table 3: Recovery response of inbred maize lines after artificial MSV inoculation

Entry	% Recovery	**Entry**	**% Recovery**	**Entry**	**% Recovery**
1	-11.25±1.25c′	36	28.33±11.67jklmnopqrstuvwxyz	71	21.43±0.00mnopqrstuvwxyza′b′
2	0.00±0.00za′b′c′	37	56.25±6.25abcdefghijk	72	16.00±4.00rstuvwxyza′b′c′
3	49.28±0.71abcdefghijklmno	38	26.51±0.42jklmnopqrstuvwxyza′	73	32.67±0.67ghijklmnopqrstuvwx
4	39.35±0.65defghijklmnopqrstuv	39	59.14±7.53abcdefghi	74	23.03±1.97lmnopqrstuvwxyza′
5	14.88±4.17rstuvwxyza′b′c′	40	53.36±9.61abcdefghijklm	75	38.73±5.39defghijklmnopqrstuv
6	15.04±6.70rstuvwxyza′b′c′	41	40.00±3.33defghijklmnopqrstu	76	12.86±2.14stuvwxyza′b′c′
7	25.73±5.04klmnopqrstuvwxyza′	42	34.74±0.26efghijklmnopqrstuvw	77	16.67±3.33rstuvwxyza′b′c′
8	39.02±2.75defghijklmnopqrstuv	43	54.38±6.23abcdefghijkl	78	20.00±0.00nopqrstuvwxyza′b′c′
9	30.50±5.50hijklmnopqrstuvwxy	44	36.93±7.90defghijklmnopqrstuvw	79	32.86±1.43fghijklmnopqrstuvwx
10	58.33±0.00abcdefghij	45	30.47±11.95klmnopqrstuvwxy	80	65.08±0.63abcdefg
11	38.93±3.93defghijklmnopqrstuv	46	51.09±1.09abcdefghijklmn	81	72.98±2.02abc
12	29.46±2.80klmnopqrstuvwxy	47	45.42±7.92abcdefghijklmnopqr	82	66.61±0.89abcdef
13	23.68±13.16lmnopqrstuvwxyza′	48	35.00±1.67efghijklmnopqrstuvw	83	75.38±0.38a
14	32.77±3.60ghijklmnopqrstuvwx	49	24.05±7.38klmnopqrstuvwxyza′	84	73.75±2.92abc
15	21.25±21.25mnopqrstuvwxyza′b′	50	73.13±0.40abc	85	66.01±0.64abcdef
16	37.32±0.18defghijklmnopqrstuv	51	0.23±5.49yza′b′c′	86	65.00±2.50ab
17	39.15±2.79defghijklmnopqrstuv	52	5.22±2.28wxyza′b′c′	87	67.44±1.13a
18	30.00±3.33klmnopqrstuvwxy	53	0.00±0.00za′b′c′	88	72.98±2.02abc
19	28.57±3.57jklmnopqrstuvwxyz	54	27.00±3.00jklmnopqrstuvwxyza′	89	73.53±2.94abc
20	48.75±1.25abcdefghijklmnop	55	28.34±1.07jklmnopqrstuvwxyz	90	69.77±1.20abcd
21	48.33±1.67abcdefghijklmnopq	56	23.57±6.43lmnopqrstuvwxyza′	91	26.18±3.45jklmnopqrstuvwxyza′
22	41.67±11.67cdefghijklmnopqrs	57	10.53±10.53stuvwxyza′b′c′	92	23.93±1.07klmnopqrstuvwxyza′
23	58.26±1.12abcdefghij	58	0.00±0.00za′b′c′	93	31.70±4.20hijklmnopqrstuvwxy
24	31.92±3.79ghijklmnopqrstuvwxy	59	45.00±5.00abcdefghijklmnopqrs	94	39.64±0.36defghijklmnopqrstuv
25	38.98±4.61defghijklmnopqrstuv	60	53.33±6.67abcdefghijklm	P-value	<.0001
26	67.44±2.93abcde	61	48.82±2.61abcdefghijklmnop		
27	-5.36±5.36a′b′c′	62	45.00±5.00abcdefghijklmnopqrs		
28	14.06±1.56stuvwxyza′b′c′	63	63.33±3.33abcdefgh		
29	7.50±2.50wxyza′b′c′	64	30.00±10.00klmnopqrstuvwxy		
30	-10.8±10.87b′c′	65	45.83±4.17abcdefghijklmnopqr		
31	13.54±5.21stuvwxyza′b′c′	66	18.34±2.71qrstuvwxyza′b′c′		
32	12.04±4.63tuvwxyza′b′c′	67	12.89±2.89opqrstuvwxyza′b′c′		
33	75.54±0.54a	68	2.50±2.50xyza′b′c′		
34	35.00±3.00efghijklmnopqrstuvw	69	14.56±1.23rstuvwxyza′b′c′		
35	7.81±1.56uvwxyza′b′c′	70	42.57±1.55bcdefghijklmnopqrs		

37 while the lowest values were recorded in maize lines 90, 44, and 75 respectively (Table 4).

The MSV virus accumulation on the maize inbred genotypes differed significantly between the top and bottom leaf upon leafhopper inoculation ($p = 0.0001$). This may be attributed to MSV virus vector behavior. MSV is primarily transmitted by leafhoppers such as *Cicadulina spp* which prefer feeding on the younger, more succulent and nutritionally rich leaves which are typically found on upper parts of the plant (Abebe, 2024). The leaves on the upper plant part are exposed and easer for insect to access compared to the older tougher leaves lower down on the plant. This feeding preference results in higher initial virus inoculation and subsequent replication in the upper leaves (Wosula *et al*. 2018).

Additionally, younger leaves mainly found on the upper part of maize plant have higher metabolic activity and more vigorous cell division. These conditions are more conducive to viral replication and accumulation. The virus can exploit the active cellular machinery for its replication effectively in these young tissues. The upper parts of the plant have the environmental condition favorable including exposure to sunlight and warmer temperatures which are conducive for the replication of the MSV

Table 4: Relative MSV Titer on Inbred Maize Lines Leaves

Maize lines	Absorbance Fold Value (AFV)	Maize lines	Absorbance Fold Value (AFV)	Maize lines	Absorbance Fold Value (AFV)
1	0.76±0.06cdefg	36	1.23±0.28cdefg	71	1.35±0.24cdefg
2	1.52±0.44cdefg	37	1.11±0.24cdefg	72	2.41±0.97abcdefg
3	0.98±0.21cdefg	38	1.06±0.18cdefg	73	0.94±0.31cdefg
4	0.98±0.16cdefg	39	0.76±0.13cdefg	74	0.82±0.20cdefg
5	3.68±1.62a	40	0.92±0.30cdefg	75	0.95±0.25cdefg
6	2.68±1.08abcd	41	1.00±0.06cdefg	76	1.95±0.86abcdefg
7	1.36±0.24cdefg	42	1.41±0.12cdefg	77	0.96±0.09cdefg
8	1.65±0.25abcdefg	43	0.90±0.20cdefg	78	1.49±0.32cdefg
9	2.57±0.53abcde	44	1.39±0.33cdefg	79	0.90±0.32cdefg
10	1.26±0.34cdefg	45	1.59±0.50bcdefg	80	0.68±0.16defg
11	1.22±0.30cdefg	46	1.44±0.36cdefg	81	0.73±0.04cdefg
12	2.49±0.97abcdef	47	0.92±0.06cdefg	82	1.34±0.43cdefg
13	2.50±0.76abcdef	48	0.74±0.08cdefg	83	1.17±0.01cdefg
14	2.13±0.45abcdefg	49	1.27±0.21cdefg	84	1.48±0.27cdefg
15	1.17±0.58cdefg	50	0.67±0.09defg	85	1.16±0.11cdefg
16	1.50±0.30cdefg	51	0.74±0.17cdefg	86	1.21±0.09cdefg
17	1.51±0.48cdefg	52	0.96±0.23cdefg	87	1.00±0.12cdefg
18	2.29±0.77abcdefg	53	1.06±0.42cdefg	88	1.14±0.07cdefg
19	2.76±1.04abc	54	1.11±0.15cdefg	89	1.02±0.13cdefg
20	0.87±0.23cdefg	55	0.40±0.71g	90	1.29±0.13cdefg
21	1.24±0.41cdefg	56	1.64±0.60abcdefg	91	2.25±0.65abcdefg
22	1.36±0.41cdefg	57	1.25±0.14cdefg	92	2.34±0.66abcdefg
23	0.50±0.06efg	58	2.23±0.37abcdefg	93	2.38±0.76abcdefg
24	0.65±0.08defg	59	0.85±0.09cdefg	94	2.36±0.76abcdefg
25	0.60±0.06efg	60	0.84±0.17cdefg	P value	P=.<0001
26	0.47±0.05fg	61	1.41±0.45cdefg		
27	1.44±0.32cdefg	62	0.74±0.13cdefg		
28	0.89±0.12cdefg	63	0.88±0.16cdefg		
29	1.49±0.43cdefg	64	1.74±0.81abcdefg		
30	0.79±0.10cdefg	65	1.97±0.90abcdefg		
31	0.67±0.17defg	66	1.30±0.35cdefg		
32	0.34±0.05g	67	3.63±1.44ab		
33	0.70±0.14cdefg	68	1.81±0.60abcdefg		
34	2.11±0.54abcdefg	69	2.13±0.48abcdefg		
35	0.70±0.25cdefg	70	1.83±0.66abcdefg		

Means with the same letters within the same column are not statistically significant at $p<0.05$ based on Tukey's post Hoc Test.

virus (Wosula *et al*. 2018). According to with Sime *et al*. (2021)using the high-throughput kompetitive allele specific PCR (KASP MSV virus inoculation results to significantly higher MSV titer on samples from upper leaves than those on the bottom leaves. Moreover, once the MSV virus is introduced into the plant by leafhoppers, it moves systemically hence higher virus accumulation on the initial site of infection (Sarwar, 2020). Also, the plant upper leaves are more active in photosynthesis, providing more susceptible resources which could potentially be exploited by the virus for its replication processes. These leaves have higher nutrient flow which could facilitate greater virus movement and accumulation (Ying *et al*. 2024).

MSV resistance of inbred maize lines based on Genotyping using selected SNP markers

The SNP markers were tested on the 94 maize lines carrying different genes associated with MSV resistance genes in chromosome 1.

Genotyping using Marker PZE-101093951

Based on the KlusterCaller genotyping, 76.60% of the tested maize lines had the homozygous gene (FAM), 17.02% homozygous for gene 2 commonly referred to as HEX florosphore while 5.32% were heterozygous for genes HEX and FAM florosphores (Plate 2).

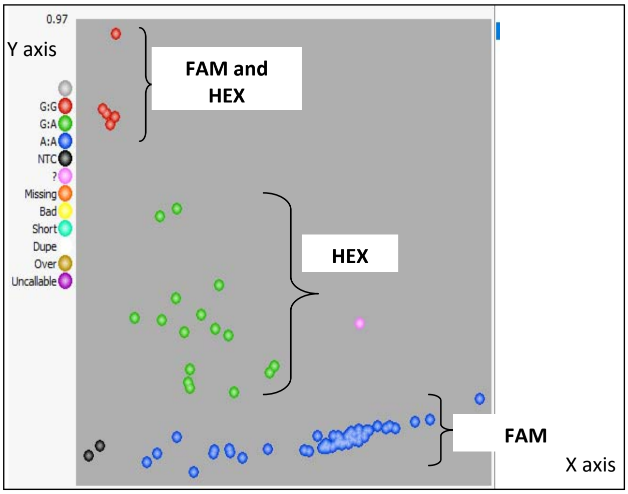


Plate 2: SNP Marker PZE-101093951 clusters showing the distribution of the HAX, HEX, and HEX and FAM alleles. Key: Red: FAM and HEX, Green-HEX, Blue-FAM, NTC-No template Control

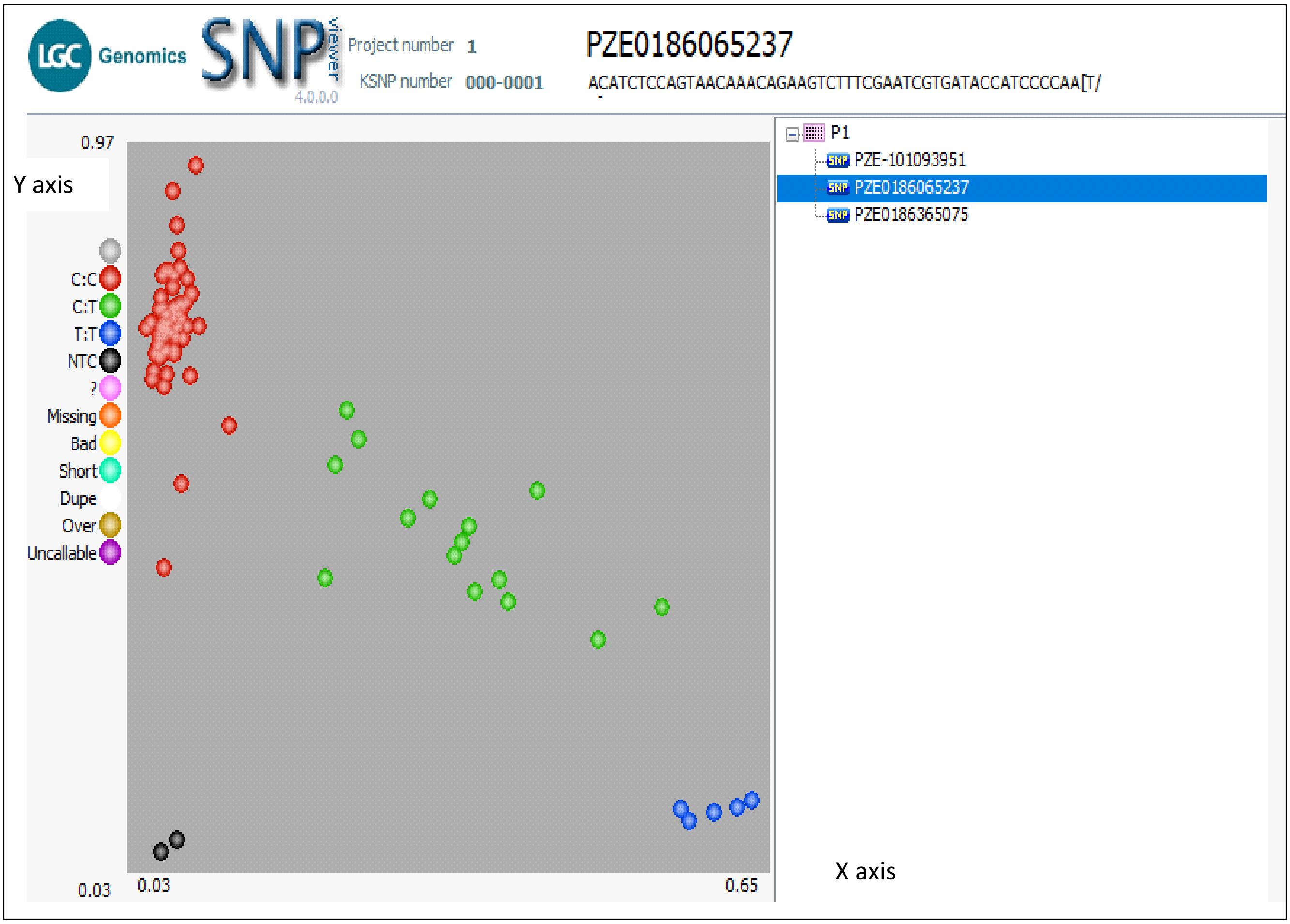
The maize lines with homozygous for HEX and heterozygous for FAM and HEX clustered on the Y axis. Clustering along the axis confirmed the presence of single allele (G:G) performance while diagonal clusters (G:A) indicated balanced heterozygosity of the allele G:A. Majority of the maize lines were homozygous for gene 1(FAM) and clustered on the X axis showing the predominance of the allele pair A:A. Therefore, 72 of the maize lines which were homozygous for gene one 1(FAM) and characterized by the allele pair A:A had common origin and were genetically distant from 16 maize lines which were homozygous for gene 2(HEX) and 5 maize lines which were heterozygous (HEX and FAM).

Genotyping using Marker PZE-101093951 showed predominance of homozygosity (AA) as represented by 72 maize genotypes. This indicates that the 72 maize genotypes had common genetic origin and derived from common ancestry line. Their clustering on the X axis reflects their genetic uniformity. In contrast, the 16 maize lines which were homozygous (GA) were genetically distinct from the homozygous (AA) indicating a different ancestry line. Moreover, the clustering of the heterozygous (GG) reflects balanced genetic contribution of both alleles and possibility of hybridization events among the homozygous maize lines. This is in agreement with Adu *et al.* (2019), where inbred maize lines clustered based on similarity in ancestry line. While the majority of the lines shared common genetic ancestry, there is significant genetic diversity within the tested maize lines, with implication for MSV resistance and breeding programs a demonstrated by (Sime *et al.* 2021). Therefore, understanding these genetic relationships is crucial for optimizing breeding strategies aimed at improving maize resistance to MSV and other agronomically important traits.

Genotyping using Marker PZE0186065237

Genotyping using Marker PZE0186065237 showed that 78.72% of the tested maize lines were homozygous for gene 1, 15.96% were positive for gene 2 while 5% were positive for the heterozygous gene (Plate 3). The heterozygous inbred maize lines reported by FAM and HEX and homozygous genotype reported by HEX clustered on the Y axis while the homozygous genotype reported by FAM clustered on the X axis. Based on this marker, the heterozygous inbred maize genotypes were from close and common genetic origin compared to the homozygous maize genotypes reported by HEX and FAM. In addition, the allele C:C was predominant (Plate 3). The allele C:T had balanced heterozygosity in the tested inbred maize genotypes.

The SNP marker showed PZE0186065237 high prevalence of allele CC which is common within the tested maize genotypes, potentially playing a significant role in MSV resistance or susceptibility. The lower proportion of allele pair CT shows that this genotype is less common among the tested maize lines but still significant and confers different level of resistance or susceptibility to MSV compared to allele CC. Despite low heterozygosity, its clustering particularly on the Y axis showed closer genetic relationship and common genetic origin compared to homozygous CC and CT. The clustering of the inbred maize genotypes based on allele shows that the genotypes have common origin. The reported heterozygosity might have risen from hybridization events between homozygous


Project number 1

KSNP number 000-0001

PZE0186065237

ACATCTCCAGTAACAAACAGAAGTCTTTCGAATCGTGATACCATCCCCAA[T/

- P1
  - PZE-101093951
  - PZE0186065237
  - PZE0186365075

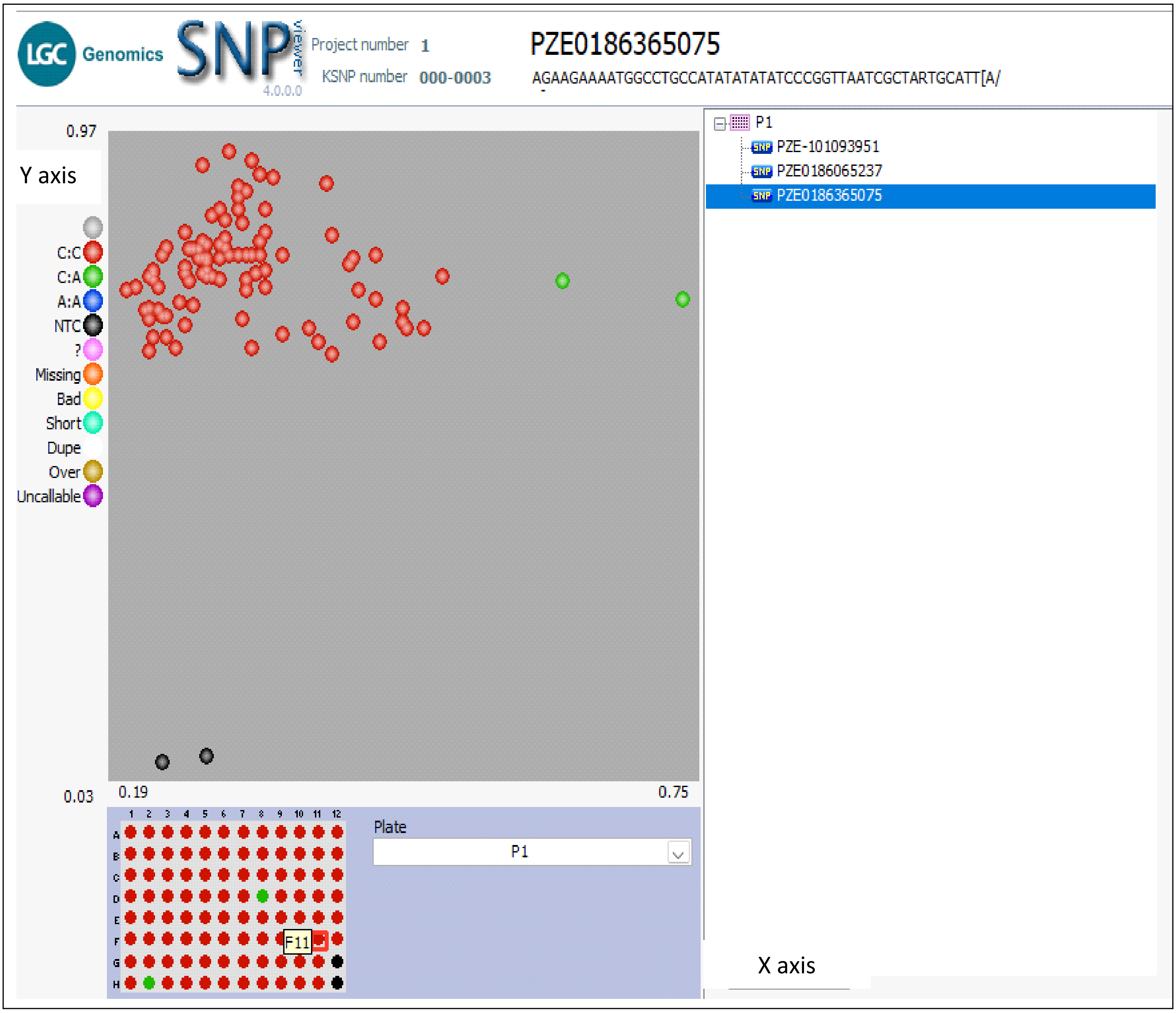
Plate 3: SNP Marker PZE0186065237 clusters showing the distribution of the HAX, HEX, and HEX and FAM alleles. Key: Red: FAM and HEX, Green-HEX, Blue-FAM, NTC-No template Control

CC and CT (Redinbaugh *et al*. 2018). The genetic diversity represented by TC allele genotypes may be leveraged to introduce new traits or enhance existing ones in future breeding efforts (Gupta *et al*. 2023).

Genotyping using Marker PZE0186365075

Genotyping using Marker PZE0186365075 showed that 97.87% of the tested maize lines were homozygous for gene 1 while 2.13 % were homozygous for gene 2 (Plate 4). The clustering of the CC allele on the Y axis showed that most of the inbred maize lines used in this study were homozygous for this allele and predominantly made of one specific nucleotide. The alternative homozygous clustered in the Y axis around 0.75 and its concentration at this point showed that they were made of the same nucleotide. Genetically, they could have close origin with the inbred homozygous maize genotypes with allele C:C (Plate 4). However, the absence of any clear clustering representing heterozygous genotype with either C or T allele suggest no inbred maize line used in this study fall into this category for PZE0186365075 SNP marker. Therefore, it suggest strong genetic differentiation at this SNP locus among the inbred maize genotypes tested for MSV virus presence.

Based on marker PZE0186365075, there was overwhelming prevalence of homozygous genotype represented by allele CC indicates that majority of the maize lines in this study shared this allele and these could be associated with trait relevant to MSV resistance or susceptibility. The dominance of this genotype suggests that the C allele is a critical component in the tested maize, potentially providing resistance to MSV or being a marker susceptibility that has been maintained through breeding. The low proportion of homozygous genotype with allele CA



Project number 1

KSNP number 000-0003

PZE0186365075

AGAAGAAAATGGCCTGCCATATATATATCCCGGTTAATCGCTARTGCATT[A/

P1

PZE-101093951

PZE0186065237

PZE0186365075

Plate P1

Plate 4: SNP Marker PZE0186365075 clusters showing the distribution of the HAX, HEX, and HEX and FAM alleles. Key: Red: FAM and HEX, Green-HEX, Blue-FAM, NTC-No template Control

indicates that this genotype is relatively rate in the tested population. However, the clustering suggests that these lines share common nucleotide which might confer different characteristics in terms of MSV resistance or susceptibility compared to the CC allele. Additionally, genotyping using this marker showed lack of heterozygosity indicates a strong genetic differentiation at this SNP locus (Patterson *et al*. 2017). Besides, it shows that the breeding program has maintained genetic purity by avoiding cross breeding that would introduce heterozygosity (Kadirvel *et al*. 2020). This approach may be utilized in preserving specific traits associated with homozygous alleles, and those potentially linked to the MSV resistance (Oliveira *et al*. 2004; Sime *et al*. 2021).

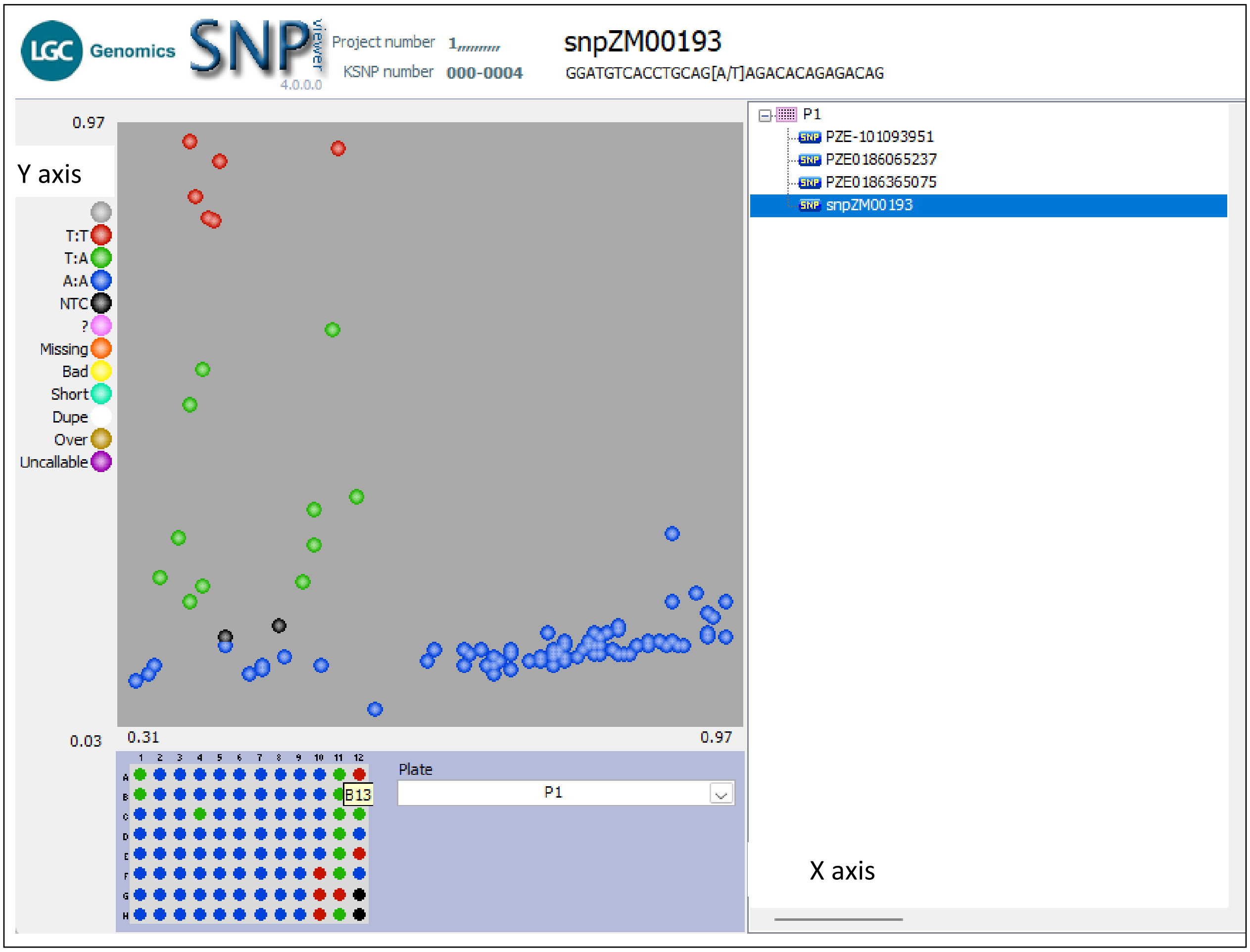
Genotyping using Marker snpZM00193

Based on the KlusterCaller genotyping, 81.91% of the tested maize lines had the homozygous gene 1 (FAM), 11.7% homozygous for gene 2 commonly referred to as HEX florosphore while 6.38% were heterozygous for genes HEX and FAM florosphores (Plate 5). The maize lines with homozygous for HEX and heterozygous for FAM and HEX clustered on the Y axis. Clustering along the axes confirmed the presence of single allele (A:A) performance while diagonal clusters (G:A) indicated balanced heterozygosity of the allele T:A. Majority of the maize lines were homozygous for gene 1(FAM) and clustered on the X axis showing the predominance of the allele pair A:A. Therefore, 77 of the maize lines which were homozygous for gene 1(FAM) and characterized by the allele pair A:A had common origin and were genetically distant from 11 maize lines which were homozygous for gene 2(HEX) and 6 maize lines which were heterozygous (HEX and FAM) (Plate 5).

The snpZM00193 marker showed predominance of homozygous gene 1(FAM) thus depicting that the breeding program focused on this genotype thus leading to genetically homogenous population based on this allele. The presence of maize lines which were homozygous for gene 2 (HEX) and heterozygous (Fam and FAM) showed that the inbred maize lines were genetically diverse. This is in line with Jagtap *et al.* (2020) where a high heterozygosity and 71% polymorphic pattern occurrence of an allele was observed in inbred maize lines and genotyped using KASP technology. This diversity is vital in breeding program aimed at improving traits such as resistance to diseases such as MSV and MLN virus (Awata *et al.* 2021a).

The KASP marker showed that different markers have the ability to distinguish various genes associated with the MSV virus. This is in agreement with previous study by Bansal *et al.* (2021) who established that different markers have different abilities of distinguishing maize inbred parental lines from the hybrid lines (Bansal *et al.* 2021). Besides, it is used to differentiate the hybrid maize lines from other plant species.

The differences in the performance and clustering of the four selected markers used on the cluster plot may be attributed to the type and the location of the degenerate base. This corroborates with previous study by Patterson *et al.* (2017) where different markers had different performance and clustering due the difference in markers ability to identify purines A or T bases or pyrimidines C and T. Use of multiple SNP in detecting various genotypes is vital in overcoming the inefficiencies associated with



Project number 1,,,,,,,,,,

KSNP number 000-0004

snpZM00193

GGATGTCACCTGCAG[A/T]AGACACAGAGACAG

Plate 5: SNP Marker snpZM00193 clusters showing the distribution of the HAX, HEX, and HEX and FAM alleles. Key: Red: FAM and HEX, Green-HEX, Blue-FAM, NTC-No template Control

one marker which may be co-inherited. Similarly, the low heterozygosity level among the inbred maize genotypes used in his study can be attributed to the effectiveness of the four SNP in forming homogenous populations (Majeed *et al*. 2023). This is in agreement with Adu *et al*. (2019) who reported that lower level of heterozygosity in inbred maize lines within sub-populations is due to effectiveness of the SNP markers in forming homogenous sub-populations.

According to Oliveira *et al*. (2004), genetic distances are used to measure the degree of relatedness between individuals in a population. The results from his study showed genetic variability among the inbred maize genotypes hence most of the inbred lines were unique and each had potential of contributing new allele for breeding MSV resistance maize lines. The SNP markers clustered the inbred maize genotypes based on their ancestry and resistance to the MSV virus. However, some of the inbred maize genotypes clustered far from others showing that despite being obtained from same source population, they may not have similar selection history as described by (Adu *et al*. 2019). The lack of association between clustering patterns and the maize genotypes heterotic groups has been previously reported by (Kadirvel *et al*. 2020). Bansal *et al*. (2021) demonstrated that the SNP markers may be better indicators of relatedness of inbred lines in cases where the inbred maize genotypes were obtained from the same source population than those obtained from different populations (Garcia-Oliveira *et al*. 2020).

CONCLUSION AND RECOMMENDATION

The severity of infection by MSV in maize lines was higher within the first two weeks however the severity reduced over time across the lines indicating their ability to resist the virus. The variability in response of maize lines to MSV may be attributed to the genetic diversity of the germplasm. The MSV accumulated more on the upper leaves than on lower leaves due to succulent, nutrition status, exposure to sunlight and warmer temperatures favouring the leafhoppers vectors. Based on the four selected SNP markers, 56 maize lines were resistant to MSV, 16 lines were moderately resistant while 22 were susceptible. The maize lines which were resistant to MSV virus to be further screened for future use in breeding programs and subsequent distribution to farmers for production.

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APPENDIX 1: Germplasm of 94 lines undergone marker-assisted backcrossing with KS-23 as a donor line

Entry	**S1 lines from backcrosses of KS23 carrying favorable and unfavorable alleles**		
1	(TZISTR1211/KS23-3/TZISTR1211)-7	20C23486-7	21A11006
2	(TZISTR1211/KS23-3/TZISTR1211)-104	20C23486-104	21A11013
3	(TZISTR1219/KS23-3/TZISTR1219)-8	20C23488-8	21A11019
4	(TZISTR1219/KS23-3/TZISTR1219)-75	20C23488-75	21A11056
5	(TZISTR1219/KS23-3/TZISTR1219)-76	20C23488-76	21A11057
6	(TZISTR1219/KS23-3/TZISTR1219)-77	20C23488-77	21A11058
7	(TZISTR1219/KS23-3/TZISTR1219)-78	20C23488-78	21A11059
8	(TZISTR1219/KS23-3/TZISTR1219)-79	20C23488-79	21A11060
9	(TZISTR1219/KS23-3/TZISTR1219)-82	20C23488-82	21A11061
10	(TZISTR1219/KS23-3/TZISTR1219)-85	20C23488-85	21A11062
11	(TZISTR1219/KS23-3/TZISTR1219)-87	20C23488-87	21A11063
12	(TZISTR1219/KS23-3/TZISTR1219)-90	20C23488-90	21A11064
13	(TZISTR1219/KS23-3/TZISTR1219)-91	20C23488-91	21A11065
14	(TZISTR1219/KS23-3/TZISTR1219)-94	20C23488-94	21A11066
15	(TZISTR1219/KS23-3/TZISTR1219)-96	20C23488-96	21A11067
16	(TZISTR1231/KS23-3/TZISTR1231)-1	20C23490-1	21A11104
17	(TZISTR1231/KS23-3/TZISTR1231)-4	20C23490-4	21A11106
18	(TZISTR1231/KS23-3/TZISTR1231)-21	20C23490-21	21A11111
19	(TZISTR1231/KS23-3/TZISTR1231)-38	20C23490-38	21A11114
20	(TZISTR1231/KS23-3/TZISTR1231)-44	20C23490-44	21A11116
21	(TZISTR1231/KS23-3/TZISTR1231)-46	20C23490-46	21A11117
22	(TZISTR1231/KS23-3/TZISTR1231)-48	20C23490-48	21A11118
23	(TZISTR1231/KS23-3/TZISTR1231)-72	20C23490-72	21A11121
24	(TZISTR1232/KS23-3/TZISTR1232)-9	20C23492-9	21A11141
25	(TZISTR1232/KS23-3/TZISTR1232)-12	20C23492-12	21A11142
26	(TZISTR1232/KS23-3/TZISTR1232)-32	20C23492-32	21A11148
27	(TZISTR1232/KS23-3/TZISTR1232)-41	20C23492-41	21A11152
28	(TZISTR1232/KS23-3/TZISTR1232)-46	20C23492-46	21A11153
29	(TZISTR1232/KS23-3/TZISTR1232)-53	20C23492-53	21A11156
30	(TZISTR1232/KS23-3/TZISTR1232)-63	20C23492-63	21A11158
31	(TZISTR1232/KS23-3/TZISTR1232)-79	20C23492-79	21A11160
32	(TZISTR1232/KS23-3/TZISTR1232)-93	20C23492-93	21A11163
33	(TZISTR1232/KS23-3/TZISTR1232)-94	20C23492-94	21A11164
34	(TZISTR1232/KS23-3/TZISTR1232)-104	20C23492-104	21A11167
35	(TZISTR1232/KS23-3/TZISTR1232)-108	20C23492-108	21A11169
36	(TZISTR1233/KS23-3/TZISTR1233)-96	20C23494-96	21A11177
37	(TZISTR1233/KS23-3/TZISTR1233)-98	20C23494-98	21A11178
38	(TZISTR1219/KS23-5/TZISTR1219)-11	20C23489-11	21A11077
39	(TZISTR1219/KS23-5/TZISTR1219)-13	20C23489-13	21A11078
40	(TZISTR1219/KS23-5/TZISTR1219)-18	20C23489-18	21A11080
41	(TZISTR1219/KS23-5/TZISTR1219)-28	20C23489-28	21A11082
42	(TZISTR1219/KS23-5/TZISTR1219)-29	20C23489-29	21A11083
43	(TZISTR1219/KS23-5/TZISTR1219)-30	20C23489-30	21A11084
44	(TZISTR1219/KS23-5/TZISTR1219)-38	20C23489-38	21A11088
45	(TZISTR1219/KS23-5/TZISTR1219)-57	20C23489-57	21A11092
46	(TZISTR1219/KS23-5/TZISTR1219)-76	20C23489-76	21A11094

47	(TZISTR1219/KS23-5/TZISTR1219)-90	20C23489-90	21A11097
48	(TZISTR1219/KS23-5/TZISTR1219)-91	20C23489-91	21A11098
49	(TZISTR1219/KS23-5/TZISTR1219)-97	20C23489-97	21A11099
50	(TZISTR1219/KS23-5/TZISTR1219)-99	20C23489-99	21A11100
51	(TZISTR1219/KS23-5/TZISTR1219)-102	20C23489-102	21A11101
52	(TZISTR1219/KS23-5/TZISTR1219)-116	20C23489-116	21A11103
53	(TZISTR1231/KS23-5/TZISTR1231)-15	20C23491-15	21A11131
54	(TZISTR1231/KS23-5/TZISTR1231)-48	20C23491-48	21A11132
55	(TZISTR1231/KS23-5/TZISTR1231)-69	20C23491-69	21A11135
56	(TZISTR1231/KS23-5/TZISTR1231)-101	20C23491-101	21A11137
57	(TZISTR1231/KS23-5/TZISTR1231)-116	20C23491-116	21A11139
58	(TZISTR1232/KS23-5/TZISTR1232)-61	20C23493-61	21A11172
59	(TZISTR1232/KS23-5/TZISTR1232)-97	20C23493-97	21A11175
60	(TZISTR1232/KS23-5/TZISTR1232)-119	20C23493-119	21A11176
61	(TZISTR1233/KS23-6/TZISTR1233)-8	20C23495-8	21A11182
62	(TZISTR1233/KS23-6/TZISTR1233)-23	20C23495-23	21A11185
63	(TZISTR1233/KS23-6/TZISTR1233)-28	20C23495-28	21A11186
64	(TZISTR1233/KS23-6/TZISTR1233)-29	20C23495-29	21A11187
65	(TZISTR1233/KS23-6/TZISTR1233)-37	20C23495-37	21A11188
66	(TZISTR1233/KS23-6/TZISTR1233)-54	20C23495-54	21A11190
67	(TZISTR1233/KS23-6/TZISTR1233)-59	20C23495-59	21A11191
68	(TZISTR1233/KS23-6/TZISTR1233)-75	20C23495-75	21A11194
69	(TZISTR1233/KS23-6/TZISTR1233)-108	20C23495-108	21A11198
70	(TZISTR1233/KS23-6/TZISTR1233)-112	20C23495-112	21A11200
71	(TZISTR1244/KS23-5/TZISTR1244)-3	20C23497-3	21A11203
72	(TZISTR1244/KS23-5/TZISTR1244)-34	20C23497-34	21A11208
73	(TZISTR1244/KS23-5/TZISTR1244)-64	20C23497-64	21A11212
74	(TZISTR1244/KS23-5/TZISTR1244)-103	20C23497-103	21A11216
75	(TZISTR1211/KS23-5/TZISTR1211)-24	20C23487-24	21A11279
76	(TZISTR1211/KS23-5/TZISTR1211)-35	20C23487-35	21A11280
77	(TZISTR1211/KS23-5/TZISTR1211)-37	20C23487-37	21A11281
78	(TZISTR1211/KS23-5/TZISTR1211)-43	20C23487-43	21A11282
79	(TZISTR1211/KS23-5/TZISTR1211)-47	20C23487-47	21A11283
80	(TZISTR1211/KS23-5/TZISTR1211)-49	20C23487-49	21A11284
81	(TZISTR1211/KS23-5/TZISTR1211)-51	20C23487-51	21A11285
82	(TZISTR1211/KS23-5/TZISTR1211)-52	20C23487-52	21A11286
83	(TZISTR1211/KS23-5/TZISTR1211)-53	20C23487-53	21A11287
84	(TZISTR1211/KS23-5/TZISTR1211)-55	20C23487-55	21A11288
85	(TZISTR1211/KS23-5/TZISTR1211)-56	20C23487-56	21A11289
86	(TZISTR1211/KS23-5/TZISTR1211)-57	20C23487-57	21A11290
87	(TZISTR1211/KS23-5/TZISTR1211)-64	20C23487-64	21A11291
88	(TZISTR1211/KS23-5/TZISTR1211)-67	20C23487-67	21A11292
89	(TZISTR1211/KS23-5/TZISTR1211)-69	20C23487-69	21A11293
90	(TZISTR1211/KS23-5/TZISTR1211)-72	20C23487-72	21A11294
91	(TZISTR1211/KS23-5/TZISTR1211)-74	20C23487-74	21A11295
92	(TZISTR1211/KS23-5/TZISTR1211)-75	20C23487-75	21A11296
93	(TZISTR1211/KS23-5/TZISTR1211)-97	20C23487-97	21A11297
94	(TZISTR1211/KS23-5/TZISTR1211)-109	20C23487-109	21A11298