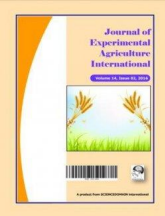

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# Quantitative Trait Loci Analysis of Maize (*Zea mays* L.) for Maize Streak Virus Resistance

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***Authors' contributions***

*'This work was carried out in collaboration among all authors. Authors PL, MW and SR designed the study and supervised laboratory and field experiments. Authors PA and PL carried out the laboratory and field experiments and wrote the first draft. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aims:** This study was conducted to evaluate partial resistance to Maize Streak Virus (MSV) in F2 segregating population derived from a single cross of two inbred lines VLO73311 (resistant) and CLRCW92 (susceptible).
**Study Design:** The experimental design was an incomplete block design replicated two times.
**Place and Duration of Study:** Department of plant sciences Kenya Agricultural and Livestock Research Institute in Biotechnology center laboratory, in June 2014 to December 2015.
**Methodology:** Resistance was evaluated in replicated field trials under artificial inoculation while selecting using SNP markers. The method of composite interval mapping was employed for QTL detection with a linkage map based on 350 SNP markers.
**Results:** The final linkage map comprised of 100 individuals and 61 SNP markers distributed in ten linkage maps and covering a distance of 437.282cM. One QTL located in linkage group four was

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detected with a LOD score of above 2.0 with two SNP markers (PZA00413_20 and PZA03198_3) tightly linked to the QTLs. A significant QTL explaining 14% of the phenotypic variance for early resistance to MSV was detected on chromosome three.
**Conclusion:** The SNPs significantly associated with MSV resistance can be used in marker-assisted selection and will accelerate the breeding process for the development of MSV resistant maize genotypes.

*Keywords: MSVD; QTLS; resistance; maize; SNP markers.*

## 1. INTRODUCTION

Maize (*Zea mays*) is the most important cereal in sub-Saharan Africa, where it is used as human food, animal feed, and raw material for various industrial products and a source of employment at various stages of production. One of the contributors to low productivity of this important crop are diseases, particularly Maize Streak Virus Disease (MSVD) which causes up to 100% yield loss in susceptible crops [1]. This large gap in yields is attributed to abiotic and biotic constraints which contribute to substantial yield losses. MSVD is caused by a virus and transmitted by leafhoppers of the genus *Cicadulina.* The disease is characterised by yellow streaks which run parallel to leaf veins. In susceptible genotypes and severe infections may result in stunting, inter-veinal necrosis, chlorosis, and death of affected individuals [2]. MSVD is difficult to control through conventional measures such as chemical, cultural, mechanical and physical methods due to its variability and unpredictable vector migratory patterns [3]. Furthermore, there exist grasses which are host reservoirs for both the insect vector and the virus [3]. It is for this reasons that this study was carried out to seek solutions leading to better management of MSVD constraint.

Development and application of MAS(Marker Assisted Selection) in crop improvement has become a useful technique for breeders. The technology has been applied for faster breeding in Kenyan maize breeding programs to introgress resistance or tolerate to biotic and abiotic stresses [4, 5]. Since MSVD resistance trait has a high heritability and is controlled by a few genes, the application of markers is quite possible and quicker to assay than in conventional breeding. This reduces the time taken to produce a variety and by extension minimises the cost, as well as the quicker availability of seed to farmers. Molecular markers can help to select individuals carrying target genes in a segregating population based on patterns of tightly linked markers rather than on their phenotypes. Also, selection of individuals carrying target genes can eliminate interactions between different loci and increase the efficiency of selection. Markers also reduce the time of selection since they are independent of growth stages of the plant as well as environmental conditions. Based on the above mentioned, the goal of this research consisted in to evaluate partial resistance to Maize Streak Virus (MSV) in F2 segregating population derived from a single cross of two inbred lines VLO73311 (resistant) and CLRCW92 (susceptible).

## 2. MATERIALS AND METHODS

Field experiments were done at Kenya Agricultural, and Livestock Research Organization, Muguga using incomplete block design replicated two times. Two maize lines were used: one resistant (VLO73311) and one susceptible line (CLRCW92). Maize plants were inoculated at three-leaf stage. For this inoculation, two viruliferous aphids (*Cicadiluna. mbila*) that had been fed on pearl millet (*Pennisetum glaucum*) infected with known MSV virus isolate insects were used.

### 2.1 Disease Assessment

MSVD severity was scored at three leaf stage at three weeks after emergence. Disease severity was scored on a 1-5 MSV disease rating scale [6,7]. This scale is adapted from the IITA, where 1 = no streaking to very light streaking (specks with no subsequent development); 2 = light streaking on old leaves gradually decreasing on young leaves; 3 = moderate streaking on old and young leaves; 4 = severe streaking on 60% of leaf area, plants stunted; 5 = severe streaking on all leaves (≥75%), plants severely stunted, dying or dead.

### 2.2 Sample Collection and Treatment

Four young leaves were sampled from each of the 100 maize seedlings per cross, at six-leaf

growth stage and separately put in small perforated bags, transported on dry ice to the laboratory and pre-chilled at -80°C overnight. The following morning, they were removed and lyophilised. The samples were frozen for 72 hours, removed and stored at -20°C. Each sample was chopped into one-inch segments and placed in a pre-chilled mortar. Liquid nitrogen was added to quickly freeze-dry the leaf material prior to grinding into a fine powder with a pestle. The ground material was put in 15ml polypropylene centrifuge tube and stored at -20°C.

### 2.3 Extraction of Genomic DNA

Genomic DNA was extracted using the sodium dodecyl sulfate (SDS) based method.

### 2.4 SNP Analysis

The normalised DNA samples were sent to KBiosciences for Single nucleotide polymorphism genotyping using the KASpar genotyping assay. Assays were prepared with 4 µL genomic DNA, 4 µL 2x KASP™ Master Mix and 0.11 µL KASP™ Primer Mix. KASP™ 2x Master Mix contained the FRET reporting system (FAM, HEX) and PCR reagents. KASP™ Primer Mixes, unique for each SNP, contained two allele-specific primers and one common primer. Both mixes were obtained from LGC. Two Triplicate assays using normalised DNA were arranged on 96-well plates (Fischer Scientific Company, Ottawa ON) with a minimum of three no-template controls and a positive control sample (‘Tardis'). Assays were carried out using a CFX96 Real-Time System with C1000 Thermal Cycler (BioRad, Hercules CA). Amplification and fluorescence reading were conducted using the following protocol: 94°C 15', 10 touchdown cycles of 94°C 20" then 65°C 1' (-0.8°C/cycle), 30 cycles of 94°C 20" then 57°C for 1', 5 cycles of 25°C 1' then plate read. Assays on the samples were carried out once. Alleles were discriminated based on normalised fluorescence readings taken at the final protocol step using CFX Manager's (BioRad) automatic calling option. Allele discrimination plots (ADPs) were confirmed and adjusted by visual inspection of fluorescence data. Alleles were recorded as 1 (FAM) or 3 (HEX). The SNP data from Kbioscience was provided as a matrix in excel and then coded and formatted into Joinmap (ver4.1) format for linkage mapping.

### 2.5 Genetic Linkage Mapping

The genetic linkage map was constructed using Join map version 4.1 [8]. The ratio of the segregation was tested using Chi-square goodness of fit. Markers that showed a lot of distortion segregation were excluded from the analysis. Linkage analysis and mapping were done using regression analysis and Kosambi mapping function [9]. The map chart software was used to compile the linkage map and locus file for QTL mapping.

### 2.6 QTL Mapping

The genetic linkage map constructed above was used for QTL analysis. The mean for the phenotypes was calculated across the replicates and used for QTL mapping. The interval mapping (IM) option of map QTL [8] was used to map the QTL intervals. A permutation test was done to determine linkage genome wise, LOD significant threshold was set at 0.05 significant levels.

## 3. RESULTS AND DISCUSSION

### 3.1 Genetic Linkage Map

Ten stable linkage groups were received with a good distribution of SNP overall groups (Fig. 1). The ten linkage groups are consistent with the ten chromosomes for maize, and thus all the chromosomes are deemed to have been covered by the markers in the linkage map.

Two of the SNP markers (PZA00413_20 and PZA03198_3) are tightly linked to the MSV trait as shown in the linkage group four (Fig. 1). The map spanned a total length of 437.282cM, in ten linkage groups (Table 1). The markers mapped predominantly on the expected position similar to the MaizeGDB maps.

In a genome-wide association study, two SNPs were significantly associated with the Maize Streak Virus on chromosome 3 explaining together 14 % of the phenotypic variance.

### 3.2 Quantitative Trait Loci (QTL) Mapping

One QTL associated with MSV was detected in linkage group four with a LOD score of 2.0 (Fig. 2). Two markers PZA00413_20 and PZA03198_3 are tightly linked to the MSV trait as shown in the QTL position. The percentage explained the phenotypic variance for the markers was 8% and 6% respectively.

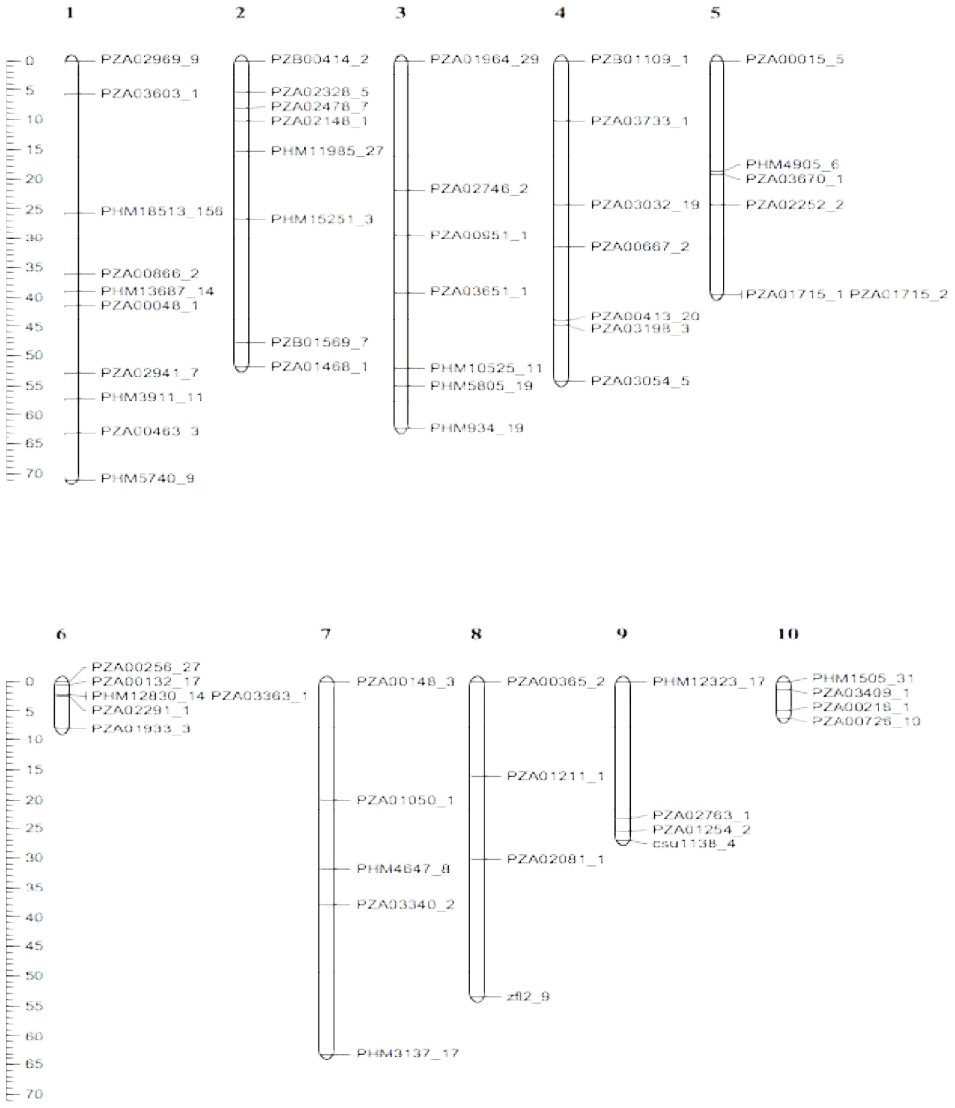


**Fig. 1. F2 genetic linkage map for maize for the cross VLO73311×CLRCW92**
**Numbers identifying the linkage group are shown on the top of each group. The names of the loci are shown on the right. Distance is shown on the left in centi Morgan**

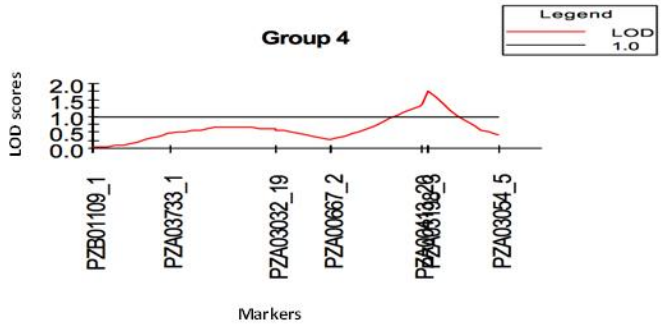


**Fig. 2. QTL associated with MSV trait in linkage group 4 with LOD threshold of 2**

**Table 1. Single nucleotide polymorphism markers mapped on 10 linkage groups**

| Linkage group | Number of markers | Length (cM) |
|---|---|---|
| 1 | 10 | 71.026 |
| 2 | 8 | 51.937 |
| 3 | 7 | 62.292 |
| 4 | 7 | 54.297 |
| 5 | 6 | 39.737 |
| 6 | 6 | 8.225 |
| 7 | 5 | 63.200 |
| 8 | 4 | 53.505 |
| 9 | 4 | 27.009 |
| 10 | 4 | 6.054 |
| Total | 61 | 437.282 |

QTL was detected with a LOD score of 2.0, the broken line indicates the LOD threshold while the red line indicates a significant QTL detected with a LOD of 2 (Fig. 2). The LOD threshold value ensured an experiment-wise error rate of $P<0.05$ in the mapping of QTL.

The QTL position was estimated at the point where the LOD score assumed its maximum in the region under consideration.

In this study, one QTL associated with MSV was identified and two markers PZA00413_20 and PZA03198_3 located in linkage group 4 were found to be tightly linked to the QTL. Two SNPs were significantly associated with the maize streak virus on chromosome 3 explaining together 14 % of the phenotypic variance indicating the possibility of the QTL being true and stable. The genetic linkage map for maize has been constructed under this study and successfully used to map the QTL associated with MSV trait and the markers associated with MSV trait identified. These results agree with results carried out in Malawi by [10]. The location of a stable major QTL of resistance to MSVD on chromosome 3 in VLO73311×CLRCW92 is consistent with the major one identified in Rev81 on the basis of flanking marker positions that border a region of 12 cM between SSR markers umc2262 and mmc2020. Nevertheless, we are not able to say whether the QTL identified in this region from Rev81 and VLO73311×CLRCW92 are allelic.

QTLs for MSVD resistance have been mapped in a cross between the maize inbred lines CML 202 (resistant) and Lo951 (susceptible) [11].Using a linkage map with 110 RFLP loci, they found four significant QTL for a disease score on chromosome 1, 2, 3, and 4, respectively, all contributed by CML202. In another study, a major quantitative trait locus (QTL) for MSV resistance was found to be on chromosome 1 in CML202 [11] a CIMMYT line, D211 [6] line from Réunion island and Tzi [12] a line from IITA. The major resistant gene was identified as msv1 in CML 202 and Tzi4.

This study was carried out to confirm the presence of this QTL and to discover any new QTLs, major or minor QTLs. The results of genotypic analysis were associated positively with [4,13]. Similar efforts, using conventional methods were initiated as early as 1930s. These efforts resulted in the identification of the first source of resistance to MSV in 1931 in the variety 'Peruvian Yellow. Later, an additional source was identified in the variety 'Arkells Hickory' which was subsequently used to develop resistant.

## 4. CONCLUSION

Based on the above mentioned, the goal of this research consisted in to evaluate partial resistance to Maize Streak Virus (MSV) in F2 segregating population derived from a single cross of two inbred lines VLO73311 (resistant) and CLRCW92 (susceptible).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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