

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

ABSTRACT

Aims: This study was conducted to evaluate partial resistance to maize streak virus (MSV) in F2 segregating population derived from single cross of two inbred lines VLO73311 (resistant) and CLRCW92 (susceptible).

Study Design: The experimental design was an incomplete block design replicated 2 times.

Place and Duration of Study: Department of plant sciences Kenya Agricultural and livestock Research Institute in Biotechnology center laboratory, between June 2014 and December 2016.

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 4 was detected with a LOD score of above 2.0 with two SNP markers (PZA00413_20 and PZA03198_3) tightly linked to the QTLs. A major QTL explaining 14% of the phenotypic variance for early resistance to MSV was detected on chromosome 3.

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.

Key words: 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 as source of employment at various stages of production. One of the contributors to low productivity of this

27 important crop are diseases, particularly Maize Streak Virus disease which causes up to 100%
28 yield loss in susceptible crops [1]. This large gap in yields is attributed to abiotic and biotic
29 constraints which contribute to substantial yield losses. MSVD is caused by maize streak virus
30 (MSV) and transmitted by leafhoppers of the genus *Cicadulina*. The disease is characterized by
31 yellow streaks which run parallel to leaf veins. In susceptible genotypes severe infection may
32 result in stunting, inter-veinal necrosis, chlorosis, and death of affected individuals [2]. MSVD
33 is difficult to control through conventional measures such as chemical, cultural, mechanical
34 and physical methods due to its variability and unpredictable vector migratory patterns [3].
35 Furthermore, there exist grasses which are host reservoirs for both the insect vector and the
36 virus [3]. It is for this reasons that this study was carried out to seek solutions leading to
37 better management of MSVD constraint.

38 Development and application of MAS in crop improvement has become a useful technique
39 for breeders. The technology has been applied for faster breeding in Kenyan maize breeding
40 programs to introgress resistance or tolerate to biotic and abiotic stresses [4, 1, 5]. Since
41 MSVD resistance trait has a high heritability and is controlled by a few genes, the application
42 of markers is quite possible and quicker to assay than in conventional breeding. This reduces
43 the time taken to produce a variety and by extension reduces the cost, as well as quicker
44 availability of seed to farmers. Molecular markers can help to select individuals carrying
45 target genes in a segregating population based on patterns of tightly linked markers rather
46 than on their phenotypes. In addition, selection of individuals carrying target genes can
47 eliminate interactions between different loci and increase efficiency of selection. Markers
48 also reduce the time of selection since they are independent of growth stages of the plant as
49 well as environmental conditions.

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52 **2. Materials and Methods**

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

59 **2.1 Disease assessment**

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

67

68 **2.2 Sample collection and treatment**

69 Four young leaves were sampled from each of the 100 maize seedlings per cross, at six-leaf
70 growth stage and separately put in small perforated bags, transported on dry ice to the
71 laboratory and pre-chilled at -80°C overnight. The following morning, they were removed
72 and lyophilized. The samples were frozen for 72 hours, removed and stored at -20°C . Each
73 sample was chopped into one-inch segments and placed in a pre-chilled mortar. Liquid
74 nitrogen was added to quickly freeze-dry the leaf material prior to grinding into fine powder
75 with pestle .The ground material was put in 15ml polypropylene centrifuge tube and stored at
76 -20°C .

77 **2.3 Extraction of genomic DNA**

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

79

80 **2.4 SNP Analysis**

81 The normalized DNA samples were sent to KBiosciences for SNP genotyping using the
82 KASpar genotyping assay. Assays were prepared with 4 µL genomic DNA, 4 µL 2x KASPTM
83 Master Mix and 0.11 µL KASPTM Primer Mix. KASPTM 2x Master Mix contained the FRET
84 reporting system (FAM, HEX) and PCR reagents. KASPTM Primer Mixes, unique for each
85 SNP, contained two allele-specific primers and one common primer. Both mixes were
86 obtained from LGC 2. Triplicate assays using normalised DNA were arranged on 96-well
87 plates (Fischer Scientific Company, Ottawa ON) with a minimum of three no template
88 controls and a positive control sample ('Tardis'). Assays were carried out using a CFX96
89 Real-Time System with C1000 Thermal Cycler (BioRad, Hercules CA). Amplification and
90 fluorescence reading were conducted using the following protocol: 94°C 15', 10 touchdown
91 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
92 cycles of 25°C 1' then plate read. Assays on the samples were carried out once. Alleles were
93 discriminated based on normalized fluorescence readings taken at the final protocol step
94 using CFX Manager's (BioRad) automatic calling option. Allele discrimination plots (ADPs)
95 were confirmed and adjusted by visual inspection of fluorescence data. Alleles were recorded
96 as 1 (FAM) or 3 (HEX). The SNP data from Kbioscience was provided as a matrix in excel
97 and then coded and formatted into Joinmap (ver4.1) format for linkage mapping.

98 **2.5 Genetic linkage mapping**

99 The genetic linkage map was constructed using Join map version 4.1 [8]. The ratio of the
100 segregation was tested using Chi square goodness of fit. Markers that showed a lot of
101 distortion segregation were excluded from the analysis. Linkage analysis and mapping was
102 done using regression analysis and Kosambi mapping function [9]. The map chart software
103 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. 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 over all groups (Figure 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 4 (Figure 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.

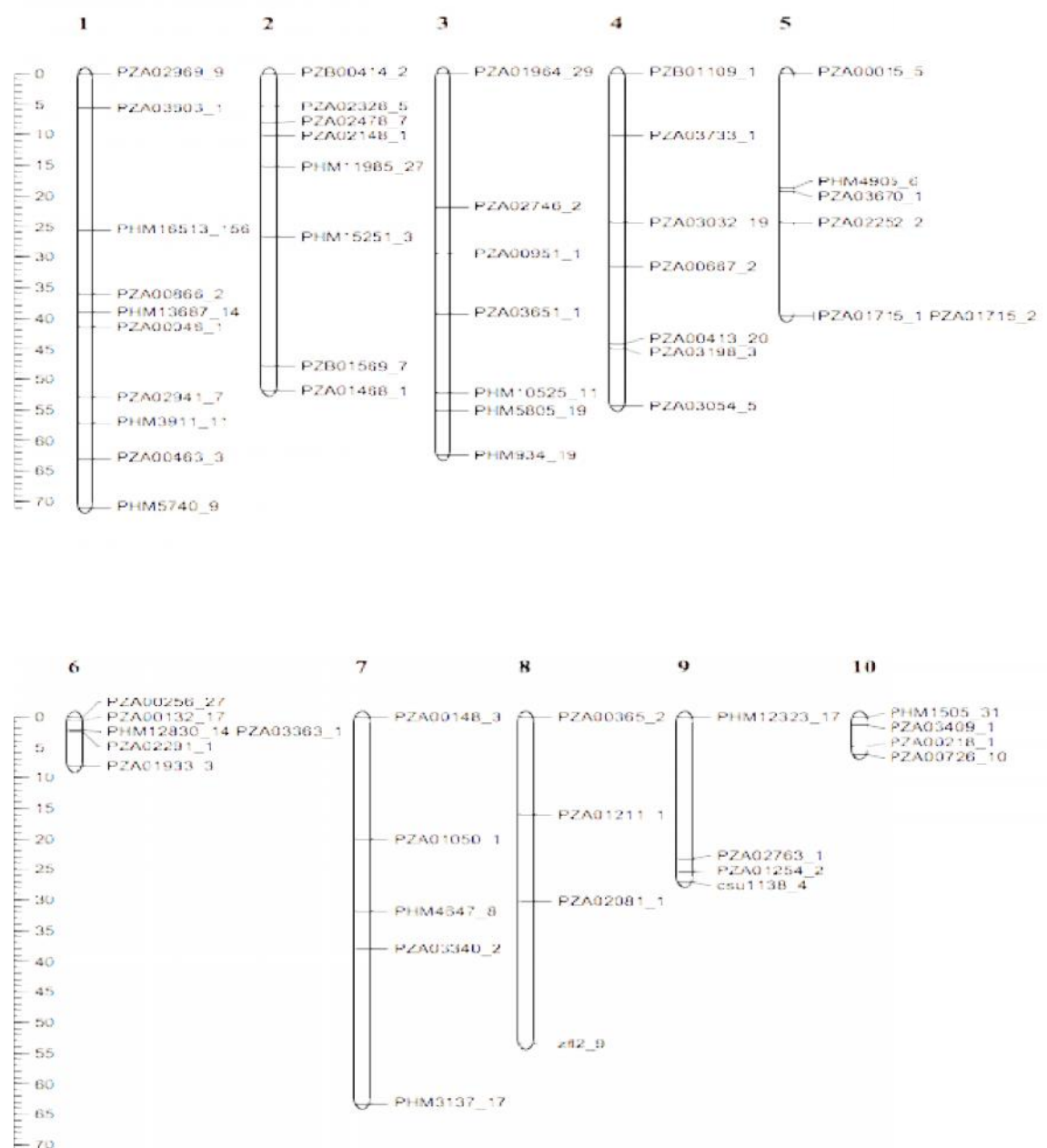
In a genome-wide association study, two SNPs were significantly associated with the maize streak virus on chromosome 3 explaining together 14 % of the phenotypic variance

Table 1: SNP 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

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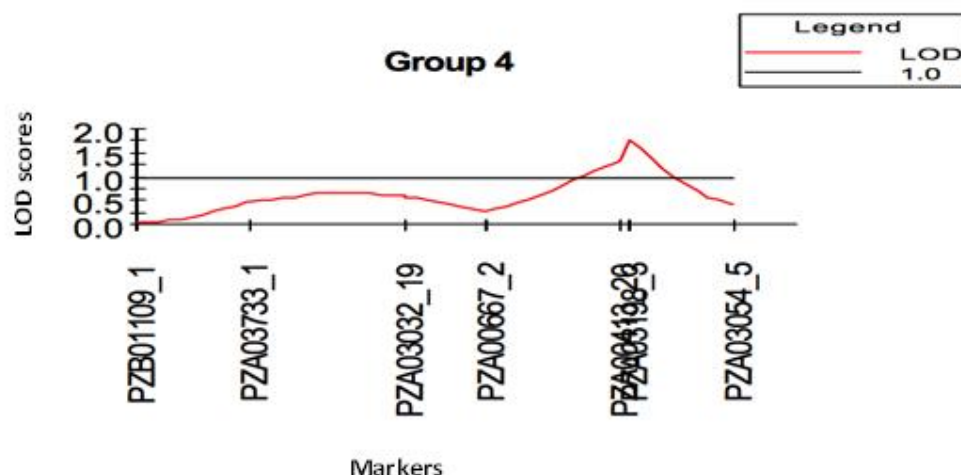
126 **Figure 1: F2 genetic linkage map for maize for the cross VLO73311×CLRCW92**

127 Numbers identifying the linkage group are shown on the top of each group. The names of the
128 loci are shown on the right. Distance is shown on the left in centiMorgan.

129

130 3.2 Quantitative Trait Loci (QTL) mapping

131 One QTL associated with MSV was detected in linkage group 4 with a LOD score of 2.0
132 (Figure 2). Two markers PZA00413_20 and PZA03198_3 are tightly linked to the MSV trait
133 as shown in the QTL position. The percentage explained the phenotypic variance for the
134 markers was 8 % and 6% respectively.



135

136 **Figure 2: QTL associated to MSV trait in linkage group 4 with LOD threshold of 2**

137 QTL was detected with a LOD score of 2.0, the broken line indicates the LOD threshold
138 while the red line indicates a significant QTL detected with a LOD of 2 (Figure 2). The LOD
139 threshold value ensured an experimentwise error rate of $P < 0.05$ in the mapping of QTL.

140 The QTL position was estimated at the point where the LOD score assumed its maximum in
141 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 designed to confirm the presence of this QTL and to discover any new QTLs, major or minor QTLs. The results from genotypic analysis were associated positively with [4, 1, 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

Molecular marker assisted selection has the potential of reducing the time it takes breeders to fix the resistance trait. This reduces the time taken to produce a variety and by extension reduces the cost, as well as quicker availability of seed to farmers.

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