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MAPPING OF QUANTITATIVE TRAIT LOCI ASSOCIATED WITH CASSAVA MOSAIC DISEASE (CMD) RESISTANCE

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Abstract: Cassava mosaic disease (CMD) is commonly considered as the most damaging disease constraint to cassava production in Africa. Identifying and breeding host-plant resistant to CMD is the most significant methods to counteract the disease and only strategy to offset the serious effect of the virus in cassava production. In this research, some landraces have been identified which exhibit high levels of resistance to CMD. However, molecular markers associated with resistance to CMD in a resistant landrace were identified, F1 progenies derived from a cross between the CMD resistant landrace TMS961089A and the susceptible line TMEB117 was initiated using 4394 SNP markers out of the 5272 derived polymorphic SNP markers from GBS were subjected to quantitative trait loci (QTL) for CMD resistance. The results revealed a single locus for CMD resistance with a strong peak LOD of 25.664cM and explaining 60% of the phenotypic variation identified on LG 15 of the map. The parent TMS961089A and nearly half of the F_I progenies were resistant to CMD severity.

Keywords: Cassava mosaic disease (CMD), single nucleotide polymorphism (SNP) and quantitative trait loci (QTL),

1. INTRODUCTION

In Nigeria, cassava mosaic disease (CMD) is one of the most important diseases of cassava, it is a major constraint to stable root production and it is associated with national or regional epidemics that flare up every few decades (Legg *et al*., 2004; Thresh *et al*., 1994). The CMD is caused by several distinct begomo virus species and the strains are transmitted by whitefly (*Bemisiatabaci*) (Gennadius) biotype A by planting of cuttings derived from disease infected plants (Brown *et al*., 1995). According to Nweke *et al*., (2002), cassava plays five important roles in African development: famine-reserve crop, rural staple food, cash crop for both rural and urban households and, to a minor extent, raw material for feed and chemical industries. Poor yields in of cassava in Africa can be attributed to a range of factors, but one of the most important is loss due to pests and diseases. The purpose of breeding for resistance is to

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improve and produce cassava cultivars that can withstand wide range of biotic and abiotic conditions. However, identifying and breeding host-plant resistant to CMD is the most significant methods to counteract the disease and only strategy to offset the serious effect of the virus in cassava production (Thresh *et al*., 1997), these resistant cultivars was obtained from breeding; which was originally obtained first from the cross between wild relative of cassava *Manihotglaziovii* and *Manihotesculenta* (Nicholas, 1947). After three backcrosses into cassava to obtain suitable storage roots, a clone 58308 was selected. Resistance in this clone 58308 has been described as recessive and polygenic (Hahn *et al*., 1980b) and for the decades this clone and its derivatives have been extensively used as the main source of resistance in breeding for resistance to the CMD disease. Using statistical methods such as single-marker analysis or interval mapping to detect associations between DNA markers and trait or phenotypic data, genes or quantitative trait loci can be detected in relation to a linkage map (Liu, 1998).

The most important use for linkage maps is to identify chromosomal locations containing genes and QTLs associated with traits of interest; such maps may then be referred to as 'QTL' (or 'genetic') maps. 'QTL mapping' is based on the principle that genes and markers segregate via chromosome recombination (called crossing-over) during meiosis (i.e. sexual reproduction), thus allowing their analysis in the progeny (Paterson, 1996). Quantitative trait loci (QTL) mapping was carried out in cassava using F1 and backcross populations, which revealed a high level of segregation (Akano et al., 2012). The studies conducted by Hahn *et al*., (1980 a, b) to establish the genetics of quantitative resistance to CMD derived from *Manihotglaziovii* indicated the possibility of several genes responsible for resistance. Inheritance study on resistance to CMD in some of the African landraces has revealed polygenic and recessive inheritance with susceptible accessions also contributing to resistance (Lokko *et al*., 1998). These genes, identified in some Nigerian landraces, confer very high levels of resistance to CMD.

The objectives of this study were to identify the inheritance of CMD resistance in a bi-parental mapping population and to constructed F_1 population genetic linkage map for QTLs linked to CMD resistance in accession TMS961089A.

2.0 MATERIALS AND METHODS

2.1SELECTION OF PLANT MATERIAL

Segregating F_1 full-sib family progenies from a cross between the breeder's accession TMS961089A and landrace TMEB117 (Isunikankiyan) were used as a mapping population in

this study. TMS961089A which exhibit resistance to cassava mosaic disease is from the earlier breeding selections derived from a cross between Nigeria landraces TME9 and TMS30572, an improved variety developed by IITA in 1973. TMEB117 is a white-rooted Oyo state, Nigeria landrace that is highly susceptible to CMD. The F_1 progenies comprising 205 individuals were used in this study, during the rainy season.

2.2 PHYNOTYPIC SCREENING

Individual plants of all genotypes were assessed at 1, 3 and 6months after planting (MAP) for their reaction to CMD under natural infection by whiteflies. CMD severity was assessed using the standard CMD scoring scale of 1 to 5 (Hahn *et al*., 1989) with CMD score of "1" classified as no visible symptom (high resistant), while those with "5" classified as very severe symptoms and stunting of the entire plant.

2.3 GENETIC LINKAGE MAPPING

After genomic DNA was extracted four weeks after emergence (germination) using the Dellaporta, *et al*., (1983) protocol with some modifications to allow high-throughput extraction of many samples. A total of 4394 SNP markers out of the 5272 derived polymorphic SNP markers from GBS were subjected to linkage analysis. Individual parental maps were developed and subsequently integrated. Linkage analysis was done following the one-step approach in Join Map 4 (Van Ooijen, 2006) using "CP option" as described in Rabbi *et al*., (2012) and previously used for other cassava linkage maps (Chen *et al*., 2010; Kunkeaw *et al*., 2010a,b; 2011). The grouping of linkage markers was evaluated using independence LOD (logarithm (base 10) of odds) values of 5 and maximum recombination frequency of 0.35. The order of markers within linkage groups was determined using regression mapping with the threshold for maximum recombination fraction and minimum LOD set at 0.45 and 3 respectively. Map distance was calculated using Kosambi mapping function (Rabbi *et al*., 2012). Final maps were drawn using Map Chart version 2.2 for the denser Map (Voorrips, 2002). Coding of the markers according to their segregation types following the Join Map notation was done using custom R script.

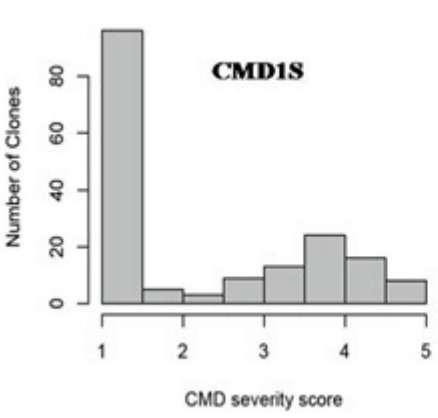
2.4 Quantitative Trait Loci Analysis

Linkage map derived from the Apek1 library that included the entire mapping population and the parents was used for the QTL analysis. Standard interval mapping was performed with the Haley-Knott regression method using the R/QTL package (Broman and Sen, 2009). Genome-wide significance threshold ($\alpha = 5\%$) for declaring QTL was determined using 1000 permutations for each trait. The proportion of phenotypic variance (R^2_{QTL}) explained by each

QTL was obtained by fitting a model including the QTLs. The effect of the detected QTLs was visualized by plotting the phenotype values versus the genotypes at a marker closest to the QTL peaks. Analysis of the QTLs was carried out using R/qtl software package (Broman *et al*., 2003).

3.0 RESULTS

The results of 205 F_1full-sib family from cross of two heterozygous clones TMS961089A and TMEB117 (Isunikankiyan) were scored for CMD disease severity on a scale of 1-5 as shown in (Fig 3.1, 3.2 & 3.3) below. The individual plants were evaluated on 1, 3 and 6 MAP in the field for their reaction to CMD under natural infection by whiteflies (*Bemisiatabaci*). The results indicated that the distribution of severity scores of 1, 3 and 6MAP followed similar trend suggesting the presence of a major gene for CMD resistance in the resistant parent.



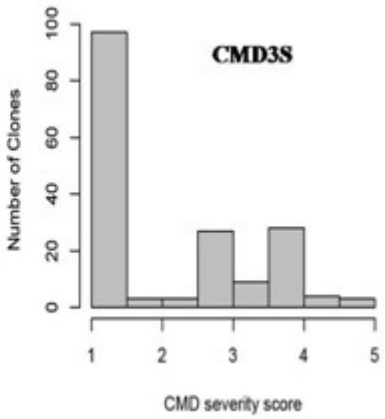


Fig 3.1: CMD Severity Score at 1MAP **Fig 3.2: CMD Severity Score at 3MAP.**

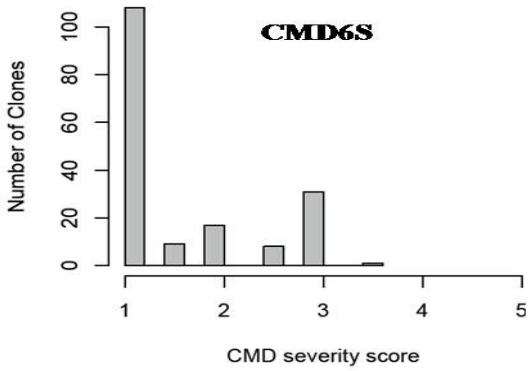


Fig 3.3: CMD Severity Score a 6MAP

A total of 4394 SNP markers that were subjected tolinkage map construction using two-step method showed group sizes ranged from 99 (LG3) to 337 SNP markers linkage group 12

with an average interval of 1.2cM between adjacent markers at a likelihood of odds (LOD) score of 3.0 and recombination fraction of 0.18 as the threshold for declaring linkage.

The QTL analysis uncovered a total of two unique genomic regions distributed across two linkage groups that control the two traits. The results revealed a single locus for CMD resistance with a strong peak LOD of 25.664cM and explaining 60% of the phenotypic variation identified on LG 15 of the map. The parent TMS961089A and nearly half of the F_I progenies were resistant to CMD severity. GBS marker from scaffold S3317_169315 at position 71.1 occurring at 250cM region of the map were most closely linked to CMD resistance gene (Figure 3.4).

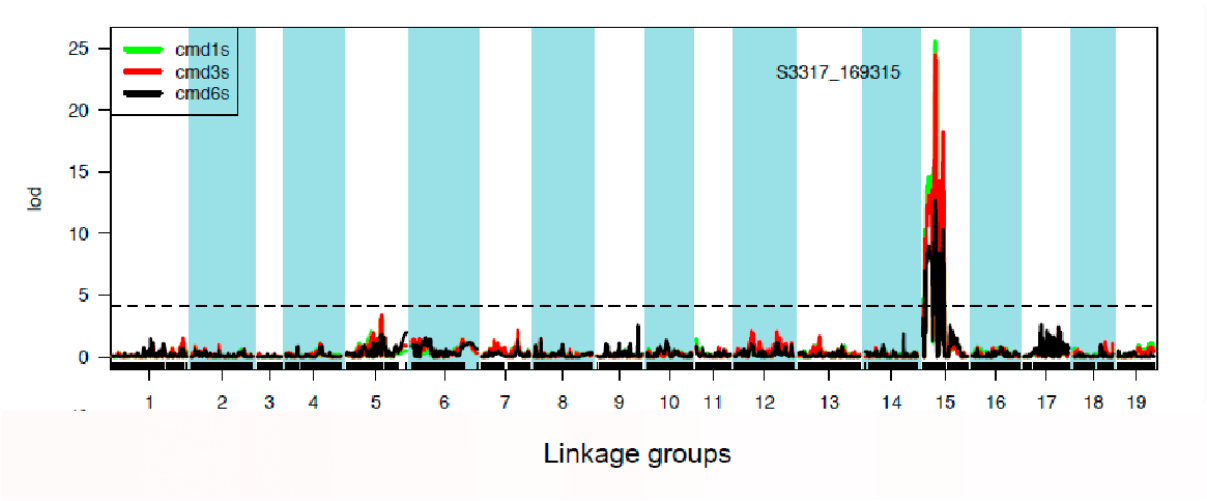


Fig.3.4: CMD Resistant QTL closely linked to SNP marker S3317_169315 at position 71.1, LG 15 with LOD 25.664cM

3.0 DISCUSSION

Cassava is an important root crop in the agricultural economies of African countries given its strategic role as a food security crop and its immense potential to grow in diverse agroecologies (El Sharkawy, 1993). Cassava Mosaic disease (CMD) is known to be major threat of cassava production in sub-Saharan Africa, breeding for resistance to CMD has been the most efficient and economically method of cassava improvement for so many years in Africa.

Therefore, the development of cassava cultivars resistant to pests and diseases, especially CMD, is vital in boosting crop productivity to enhanced income and improved livelihood of farmers in sub-Saharan Africa as well as serving as the engine that can accelerate rural development and growth in farming communities. Prior to the discovery of the single gene resistance by Akano *et al*., 2002, the primary defence against the disease was the polygenic resistance introgressed into cultivated cassava (*M. Esculenta* Crantz) from *M. glazioviia* after

three cycles of backcrossing (Nicholas, 1947). However, these descendant is immune to infection by cassava mosaic germiniviruses (CMGs), although some express mild and sometimes transient symptoms as a result of incomplete systematic infection that leads to reversion of symptoms while other are quite susceptible to the disease (Fargette *et al*., 1994). In the present study, crosses were made between resistant (TMS961089A) and susceptible (TMEB117) clones and the frequency distribution of the CMD severity incidence scores in the mapping population revealed a bi-modal pattern of distribution with two peaks. Nearly more than half of the progenies (60%) were resistant to CMD and showed no symptoms while the remaining F_1 progenies (40%) showed disease symptoms with African cassava mosaic viruses (ACMV) ranging from mild (2) to severe (5).

These results were in agreement with the finding of Ariyo, (2004) also confirmed that ACMV in single infection was the most predominant causal agent of cassava mosaic disease (CMD) in IITA experimental fields under study at all locations. In this present study, genotype-by-sequencing (GBS) was used to simultaneously discover and generate large number of SNP markers. The resulting evaluation of high-density SNP markers were applied to develop 19-linkage groups genetic map with extensive genome coverage to map QTLs controlling CMD resistance.

The number of markers on each linkage group ranged from 99 on linkage group 3 to 337 on linkage group 12, with a total map distance of 5929centi Morgan (cM), and an average marker interval of 1.2 cM, indicating that the SNP markers used were adequate to sufficiently give a dense map. This because the dense the map, the more likelihood of detecting significant QTLs that are tightly linked to CMD resistance. This result is in agreement with previous studies by Rabbi *et al.* (2012), who reported the first SNP-based genetic linkage map containing 568 markers distributed across 19 linkage groups. This result also agrees with those of Ahn and Tanksley (1993) and Lander and Botstein (1989) that the efficiency of marker-assisted breeding depends on the degree of saturation of genetic maps.

5.0 Conclusion

The use of SNP markers to generate linkage map and QTLs responsible for CMD resistance is efficient. However, the need to further investigate CMD resistance gene using other molecular markers will help to ascertain the authenticity of QTL in identifying the CMD resistance cassava varieties through marker assisted selection. This research has shown that resistant genotype TMS961089A is a CMD resistant breeding variety.

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