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Responses of Bi-Parental Mapping Population to Cassava Green Mite (CGM), Cassava Bacteria Blight (CBB) and Other Important Agronomic Traits

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Abstract
Several biotic factors constrain cassava production in the cassava-cultivating regions of Africa. In Nigeria for instance, cassava bacterial blight (CBB) and cassava green mite (CGM) are among the major constraints that cause significant yield losses to cassava growers. Cost-effective mitigation measures for these constraints include adopting and using improved resistant varieties. In this study, 262 progenies derived from a bi-parental cross were evaluated at Otobi, Benue state Nigeria to determine their responses to CBB, CGM and other important constraints. The experiment was laid out in an alpha lattice design with two replications. Results revealed that CBB and CGM severity scores at 3, 6 and 9 months did not vary significantly among the assessed clones. However, CBB incidences at three and nine months varied significantly ($p<0.05$) among the genotypes. CGM incidence also varied significantly ($p<0.01$) among test genotypes. CBB mean severities ranged from 1.6 to 2.2, and the mean severity for CGM was 2.01. Plot-based broad-sense heritability estimates for CBB severity ranged from 0.01 to 0.04, while CBB incidence ranged from 0.08 to 0.11. The genotypes also differed markedly ($p<0.001$) for specific gravity and dry matter, with mean values of 1.12 and 35.3 respectively. After the assessment, twenty clones were selected using a non-weighted summation selection index for further screening. It is expected that the study will aid in the development of CBB and CGM-resistant/tolerant cassava genotypes, for cultivation in the guinea savanna region of Nigeria.
Keywords: Cassava, Bacterial blight, green mite, dry matter and heritability

Introduction

In sub-Saharan Africa, cassava (*Manihot esculenta* Crantz) is a crucial crop for food and economic security. Due to growing population pressure, it is projected that the demand for cassava will rise during the coming decades. Nigeria is the world's top producer of cassava, and by 2030, a production shortfall of 12 metric tons is anticipated (IITA, 2017). Because, since the yield of cassava storage roots has not improved much in the majority of regions during the 1990s, there are worries about future cassava yield shortages (Ceballos *et al*., 2016), with yields declining by 0.024 t ha^{1} per year (FAO, 2016). Cassava is prone to devastating fungal, bacterial and viral diseases of mycoplasma origin (Lozano *et al*., 1981; Theberge, 1985), such as cassava green mite (CGM) and cassava bacterial blight (CBB) etc. One of the most serious diseases of cassava is cassava bacterial blight (CBB), which affects many regions where the crop plays a key role in both economic and gastronomic needs. CBB is present in all cassava-growing regions of the world. The rainfall season marks the time epidemics arise as humid conditions and warm temperatures promote the spread of these germs and trigger the emergence of symptoms. CBB causes a variety of symptoms, mostly affecting leaves, petioles, and stems, which eventually causes the plant to wither and die. Brown to dark-brown patches on the tissue of the leaves are the first signs of CBB. Bacteria that enter the xylem vessels from the mesophyll and traverse towards the stem through the petioles as the disease worsens cause the leaf to wilt (Zarate-Chaves *et al*., 2021). It could damage the crop more than any other bacterial disease and is the second most deadly disease after CMD. CBB results in significant losses of both planting material and fresh root yield (Terry, 1997). The most significant mite species causing a reduction in cassava root yield is the cassava green mite (CGM), which is caused by M. *tanajoa* and is native to the Neotropics. CGM has a colour ranging from green to yellowish and is rarely visible to the unaided eye (Yaninek and Hanna, 2003). They feed by sucking and burrowing the plant host. They suck out the fluid from palisade and spongy mesophyll cells by inserting their chelicerae (stylets) into the abaxial surface of cassava leaves (Yaninek *et al*., 1989). Chlorosis is the result, and it progresses from a small amount of pale to yellowish look to a full loss of green pigmential tissue (Bellotti *et al*., 2012). Heavy CGM infestations can result in

defoliation starting from the apical tip of the plant and lateral buds down to the shoots, producing severe candlesticks and frequently leading to dieback. By decreasing the plant's leaf area, CGM slows the plant's development rate and ability to photosynthesize (Tomkiewicz *et al*., 1993). Damage from the mite promotes weed infestation and root rot disease in cassava, and it reduces the amount and quality of planting material (Yaninek *et al*., 1989). In the Neotropics and Africa, yield losses brought on by CGM have been estimated to range from 10 to 80% (Yaninek and Hanna, 2003). CGM-induced losses are more severe when the dry season is longer (3 to 6 months) and less severe when the dry season is shorter (Byrne *et al*., 1983). Improved varietal resistance has become the most effective and efficient method of managing biotic stresses in cassava (Ogbe *et al*., 2003). Therefore, the most cost-effective long-term strategy for sustainable cassava production is thought to be the availability of high-yielding and pest and disease-resistant cultivars to farmers (Kueneman 2002). The goal of this study was to assess bi-parental cassava progenies that were resistant to cassava bacterial blight. (CBB) and Cassava green mite (CGM) and identify the relationship between diseases and yield components. The study objective is to develop cassava genotypes with high and durable resistance to CBB and CGM.

Materials and Methods

Planting materials: Two hundred and sixty-two (262) F1 progenies derived from a bi-parental cross with their two parents (TMS 97 2005 X TMS 30555) were evaluated under natural field conditions for their reaction to CBB and CGM. The parent lines were sourced from the cassava research program NRCRI. TMS 97 2005 is resistant to CBB, CGM and other diseases of cassava and was used as a female parent stock while TMS 30555 a susceptible and infectious to cassava diseases was used as the male parent for hybridization. The two cross combinations yielded reasonable progenies that were cloned for field evaluation at the clonal stage at Otobi, the major cassava growing belt for assessment for resistant genotypes to CBB and CGM. Field preparation and experimental layout: The experiment was carried out at (Guinea Savannah). The land preparation was done by ploughing, harrowing and ridged by a tractor. The experiments were laid out in alpha lattice design with two replications and the plot size used was 5m x 4m (20m2). The two parent lines were used as check/ control, the susceptible genotype and Ichenke, a susceptible farmer's variety were planted in every block of the trial as spreader row to infect the clones being evaluated and ensure there was the presence of the disease vector carrier. A total land area of 0.8ha was used. Pre and post-emergence herbicides were sprayed immediately after planting. First, second and third weeding operations were carried out by manual hoe weeding. Fertilizer, NPK 15:15:15(400kg/ha) was applied six weeks after planting. Data were collected on growth, pests/diseases. Disease incidence/severity was scored at 1, 3 6 and 9 months after planting following cassava-base ontology. Harvest data and yield components were collected to evaluate the effect of these diseases on fresh root yield. Phenotypic data obtained showed different reactions and four disease response profiles were observed among the clones and these include; (i) No disease occurrence (ii) Mild occurrence (iii) Average occurrence (iv) severe occurrence. Data sets generated were analyzed using R statistical software (R Core Team, 2020). Pearson's correlation test was carried out, and the *cor.* test was used to retrieve the coefficient of correlation. All of the aforementioned functions were accessible using the R package ggpubr. The lme4 tool in R was used to estimate the variance components, which allowed for the estimation of the best linear unbiased predictions (BLUPs). For the unbalanced dataset, the BLUPs were generated to allow genotype comparison. The following linear model was used to generate the analysis of variance for single-site analysis:

$$Y\_ijk=\mu+C\_i+B\_j+G\_k+\varepsilon\_ijk$$

Where: Y_ijk is the observed response of the i-th clone on the j-th block and k-th test genotype, μ is the general mean of the genotypes, C_i is the fixed effect of the checks, B_j is the random effect of the block, G_k is the random effect of the test genotype, ε_ijk is the random error associated with ijk-th observation. The broad-sense heritability for each trait was calculated using the formula:

$$H^2 = [\![\sigma]\!]^2 G/(\sigma^2 G+\sigma^2 e/r)$$

Where H^2 is the broad-sense heritability σ ^2 G is the genotype variance, r is the replication, and σ^2 e is the error variance. To extract variance components for computing broad-sense heritability, block and test-genotype effects were considered random, while check effects were considered fixed. A non-weighted rank summation selection index (SI) was used to compare the performance of test genotypes. Briefly, genotype BLUP values generated from yield and defensive traits were ranked and subsequently summed up to compute the selection index.

Results and Discussion

Results

Response of cassava genotypes to CGM and CBB

A total of 262 genotypes were utilized for the clonal experiment during the 2018-2019 season. CGM severity scores ranged from 1 to 5, with a mean score of 2.01 (Table 1). The percentage of CGM incidence ranged from 0 to 100. For CBB at three, six and twelve months, severity scores also ranged from 1 to 5. Around 66% of the test genotypes had a severity score of 1 and an incidence score of 0, for CBB at 3 months (Figures 1A and B). The same trend can be reported for CBB at 9 months after planting. On the other hand, only 23% of the test genotypes showed no symptoms of CBB at 12 months. For CGM, around 48% of the genotypes

showed zero incidence and no symptoms at the time of evaluation.

Variation, heritabilities for CBB, CGM and other important traits

Analysis of variance (ANOVA) results showed that there were no significant differences among test genotypes for CBB severity at 3, 9 and 12 months (Table 1). On the other hand, there were significant differences among test genotypes for CBB incidence at 3 ($p \leq 0.05$), 9 ($p \leq 0.05$) and CGM incidence ($p \leq 0.01$). There were also significant differences among test genotypes for dry matter ($p \leq 0.001$). FYLD and DYLD did not differ significantly among the test genotypes. Broad sense heritability estimates were generally low for disease traits, ranging from 0.01 to 0.11 (Table 1). For the agronomic traits, heritability estimates ranged between 0.01 to 0.15. CBB12s had the lowest heritability estimate for the disease traits, with H^2 of 0.01. For the agronomic traits, DM had the highest H^2 estimate of 0.15.

Correlation between CBB, CGM and other important traits

Correlations between CBB, CGM and other agronomic traits are presented in Table 2. Correlations between CBB12s and FYLD were negative and significant (r = 0.34). A significant negative correlation was also noted between CBB12s and DYLD (r = 0.35). There was a significant positive correlation between CBB3i and CGMi (r = 0.41). DYLD also correlated significantly with FYLD (r = 0.98). A positive significant correlation was also noted between HI and FYLD (r = 0.46)

Comparisons and Ranking of Cassava Clones Based on the Selection Index

Genotype ranking based on the non-weighted rank summation SI revealed the overall best performer as 214D (Table 3). Other top five performers included 069D, 033D, 073D, and 023D. These genotypes performed better than the check TMS972205. Another check, Ichenke, was among the worst 15 performers. The overall worst performer was the genotype 080D.

Discussion

CBB and CGM are among some of the most important diseases of cassava in Nigeria (Legg & Alvarez, 2017), undermining investments made to yield revenue from cassava production. For this reason, efforts are being put in place towards finding sustainable disease management options. Accordingly, the study aimed to contribute towards developing improved cassava varieties with enhanced CBB and CGM resistance in Nigeria. Consequently. 262 genotypes were evaluated for their responses to CBB and CGM. Results showed that 66% of the genotypes were symptomless and free from CBB incidence. That number reduced from 66% to 23% by the 12th month, thus highlighting the possibility of bacterial load increment in the test genotypes. This increment can be a result of continuous interactions of test genotypes with whiteflies, which are vectors that transmit the disease. Findings by Maruthi *et al*. (2017) revealed how whitefly aids in the spread of cassava diseases. Disease inoculum in stems can also build up due to subsequent recycling of cassava stems. Low mean CGM incidence and severity exhibited by test genotypes suggest that the clones possess resistance alleles for the disease. There were no variations among test genotypes for both CBB and CGM (Table 1). Low mean scores indicate that most genotypes exhibited low symptoms, explaining why there were no significant variations among test genotypes, a phenomenon also reported by Ano *et al*. (2021). For the agronomic traits, the means of DM and FYLD were comparable to findings by Njoku and Mbah, (2020). Broad sense heritability estimates for all traits were generally low (Table 1).

The low estimates again point towards the low genetic variation exhibited among the test genotypes for the reported traits. Low H^2 could also signify that the variations in our test genotypes are largely due to the environment, or that our traits are not highly heritable from parents to offspring. This further denotes that the traits cannot be improved further by simple phenotypic selection among the genotypes, a phenomenon also noted by Ntawuruhunga and Dixon, (2010).

Significant negative correlations between CBB12s and FYLD (Table 2) illustrate the damage done by CBB, and the need to continue breeding for cassava varieties that are tolerant/resistant to the disease (Fokunang *et al*., 2000). Significant positive correlations between CGM, CBB3s and CBB9s (Table 2) suggest a synergistic interaction between the two diseases, as previously observed between CBB and cassava anthracnose disease (CAD) (Fokunang *et al*., 2000). The non-significant positive correlations between CGMi, DYLD, FYLD and DM (Table 2) were likely due to high levels of CGM resistance in the test genotypes, or due to low disease pressure during the evaluation period.

A non-weighted rank summation SI, which comprised the summation of genotype rank for all traits, was used to rank the performance of the test genotypes (Table 3). Of the 15 selected genotypes, 214D (SI = 401) was the overall best, exhibiting marginal disease symptoms whilst maintaining high yields. Other genotypes such as 069D (SI = 445), 033D (SI = 729), and 073D (SI = 908) also performed well for both disease and yield traits. Seven test genotypes outperformed the check TMS972205 (Table 3). TMS972205 is known to have multiple pest resistance, high yielding (31.8 t/ha) and suitable for food, industry and livestock feed (Dixon *et al*., 2005). Overall, these genotypes identified to be best performers merit further investigations to confirm their resistance status, especially for CBB and CGM.

Conclusion

This study was set up to determine the field reaction of 262 test genotypes to CBB and CGM. The two diseases are of importance to Nigerian farmers. Therefore, developing clones that are resistant to them is of great value. Based on generated datasets, we identified 15

promising genotypes with dual resistance to CBB and CGM. These genotypes can be re-evaluated at higher plot capacities and in diverse sites to inform their resistance/tolerance to said diseases. These clones will help the breeding program to develop varieties with more durable resistance to the disease, which when deployed, will help in effective control of the disease spread and reduction in associated yield losses.

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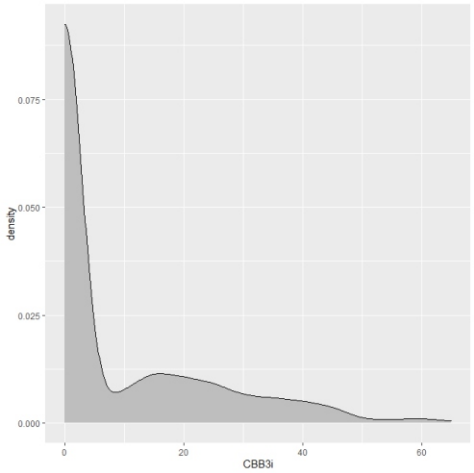


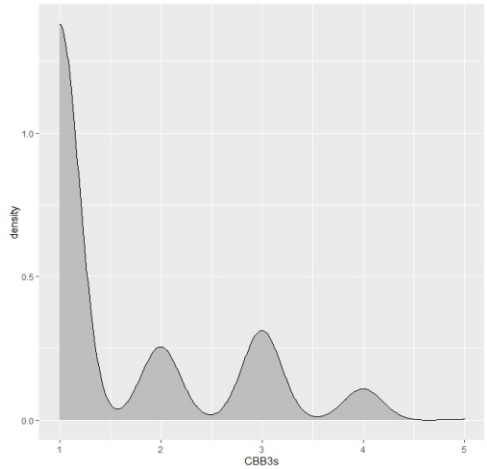
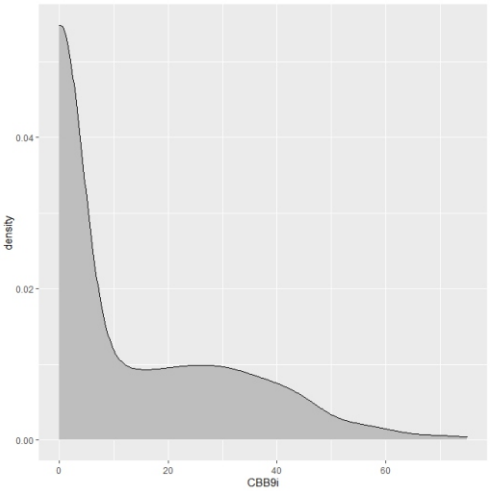


Fig 1A. CBB incidence distribution at 3MAP ***Fig 1B. CBB severity distribution at 3MAP***



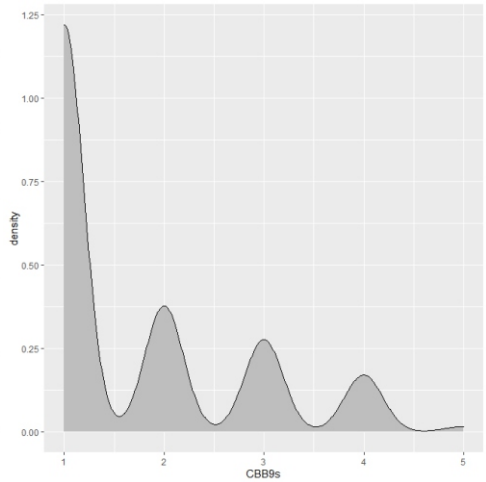
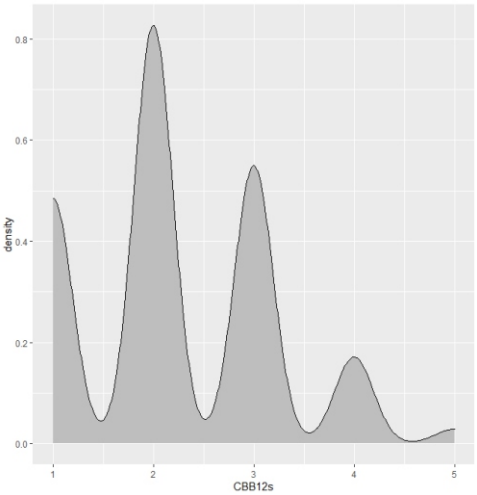


Fig 1C. CBB incidence distribution at 9MAP ***Fig 1D. CBB severity distribution at 9MAP***



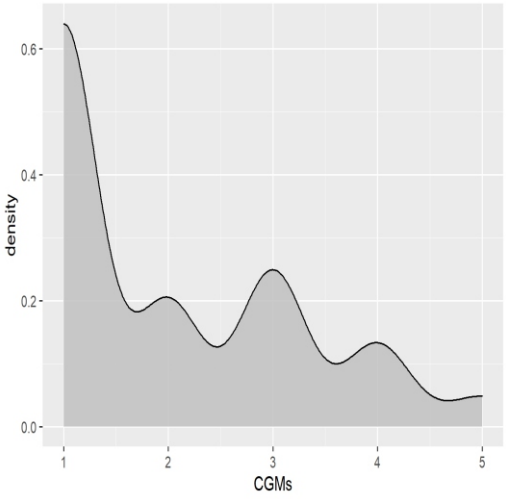


Fig 1E. CBB incidence distribution at 12MAP ***Fig1F. CGM severity distribution***

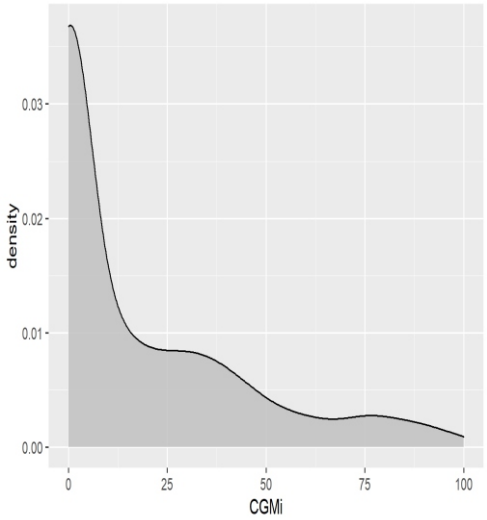


Fig1G. CGM incidence distribution