

Full Length Research Paper

Effect of mosaic virus diseases on dry matter content and starch yield of five cassava (*Manihot esculenta* Crantz) accessions in Ghana

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The effect of mosaic virus diseases on dry matter content and starch yield of five local accessions of cassava, "Ankrah", "AW/17", "Tomfa", "Dagarti" and "Tuaka" was evaluated. Tomfa showed the highest (95%) incidence of the disease, index of severity of symptoms for all plants (ISS_{AP}) of 3.70, as well as, for diseased plants (ISS_{DP}) (3.84) while Dagarti did not show any phenotypic expression of the disease throughout the study period. Most of the accessions displayed mosaic disease symptoms two months after planting but by the fifth month had fully recovered. However, polymerase chain reaction (PCR)-based testing at 12 months after planting revealed the presence of ACMV in all the accessions while EACMV was observed in Ankrah, Dagarti and AW/17. Mean tuber (fresh root weight) and starch yield at 12 months after planting (MAP) was significantly ($P \leq 0.05$) high in Ankrah while percentage dry matter was significantly higher in Dagarti than the other accessions. A negative correlation between starch yield and cassava mosaic disease incidence implies that a high mosaic incidence particularly in the first three months results in lower tuber and starch yields.

Key words: Mosaic virus diseases, dry matter, starch yield, PCR, disease incidence.

INTRODUCTION

In spite of the adaptability of cassava to edaphic and climatic factors, its average yield is only about 20% of the yield under optimum conditions (Pellerin, 2008). This poor yield is largely due to pests and diseases which account for about 90% of the losses (Wydra and Msikita, 1998). Cassava mosaic disease (CMD) is the most severe and common disease in sub-Saharan Africa (Alabi et al., 2011) and according to Legg and Thresh (2004), it is one of the most damaging plant viral diseases worldwide. Total yield losses as a result of CMD infection

in Africa have been estimated at 12 to 23 million tonnes, representing 15 to 24% of total cassava production (Thresh et al., 1997). The damaging effects of the disease are chlorotic yellowish blotches with frequent leaf deformations which result in premature leaf abscission or stunting of plants in severe cases. Dual infection with two different cassava mosaic geminiviruses (CMGs) cause severe damage (Fondong et al., 2000; Owor et al., 2004) and could undermine the effectiveness of breeding programmes aimed at producing virus-resistant propa-

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gules for farmers (Thresh and Cooter, 2005).

A general relationship between CMD symptom severity and vegetative growth of cassava has been observed in several field experiments (Fokunang et al., 2001; Tumwesigye et al., 2006). A negative correlation between CMD infection and starch yield in Ugandan cassava varieties was reported by Alves (2002). The incidence and severity of CMD usually reach maximum levels in the first three months after planting, which is also the most critical stage of vegetative growth and development. Thus, a reduction in leaf size as a result of CMD infection affects dry matter partitioning from the leaves (source) to storage roots (sinks), resulting in yield loss. Correspondingly, Mariscal et al. (2002) observed that a reduction in cassava leaf area resulted in reduced tuber bulking rate and yield.

There are only few reported studies on the effect of CMD on dry matter content and starch yield in cassava. Yet dry matter and starch yield are two important parameters which affect the use of cassava both as food and an industrial raw material (Hahn, 1989). Moreover, in Ghana, the effect of CMD on starch and dry matter content is often overlooked since most farmers can still harvest some tubers even under severe CMD infection. Therefore, the effect of CMD on dry matter content and starch yield for five accessions of cassava, Ankrah, "AW/17", "Tomfa", "Dagarti" and "Tuaka" in southern Ghana was investigated in this study.

MATERIALS AND METHODS

Symptom severity and disease incidence

Five cassava accessions namely "Ankrah", "AW/17", "Tomfa", "Dagarti" and "Tuaka" were planted in a randomized complete block design in three replicates in a field at BNARI, Accra. The cassava cuttings used were indexed for ACMV/EACMV prior to planting via Polymerase chain reaction (PCR). Cassava mosaic disease (CMD) severity was scored by visual observation of young leaves using a five (5) point scoring scale described by Njock and Ndip (2007). Index of severity of symptoms for all plants (ISS_{AP}) as well as for diseased plants only (ISS_{DP}) was calculated as described by Dellaporta et al. (1983). Disease incidence (DI) was also estimated as the number of infected plants over the total number of plants expressed as a percentage.

DNA extraction and PCR detection of ACMV and EACMV

Total DNA was extracted from young cassava plant leaves four weeks after planting using a modified procedure (Ndunguru et al., 2005). Fresh leaf samples were submerged in absolute alcohol instead of liquid nitrogen for 24 h to stop enzymatic reaction prior to DNA extraction. Polymerase chain reaction (PCR) amplification was carried out in a 25 μ l reaction mixture containing 7.5 μ l ready-mix PCR buffer without $MgCl_2$, 5.0 μ l of 25 mM $MgCl_2$, 1.0 μ l (10 μ M) each of forward and reverse primers and 2.0 μ l of template DNA. Oligonucleotide primers used were JSP001/JSP002 which detects African cassava mosaic virus (Fondong et al., 2000), EAB555F/R for detection of East African cassava mosaic Virus (EACMV) (Ndunguru et al., 2005) and UV-ALIF/ACMV-CPR3 for detection of

the Ugandan variant of EACMV (Alabi et al., 2008; Sambrook et al., 1989). The reaction was performed in a 96-well Eppendorf Thermal Cycler (Eppendorf AG, Hamburg). The thermal cycling conditions used were an initial denaturation step at 94°C for 2 min followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 58°C for 1 min and an elongation at 72°C for 1 min. These were then followed by a final elongation step at 72°C for 10 min.

Gel electrophoresis and PCR products

Gel electrophoresis was done on 1.0% agarose gel according to Murphy et al. (1991). Ten microliter (10 μ l) aliquots of the PCR products were mixed with 2 μ l of loading dye (Bromophenol blue-Sigma) and loaded into the wells and run at 90 V for 1 h on 1% agarose gel. The gel was visualized under high performance ultraviolet transilluminator (UVP, Cambridge, UK) and images captured with the aid of UVP Life Sciences Software (Doc-It LS Image Acquisition).

Tuber number and yield

Five plants of each accession were harvested 12 months after planting. Using the crop cutting method of Hedge and Hofreiter (1962), the number of tubers as well as weight per plant was recorded. Tuber yield was expressed in terms of fresh root weight.

Determination of starch yield

The skin of harvested cassava tuberous roots was peeled and tuberous roots rinsed. Approximately 10 kg of each accession was weighed and milled using a mill (Hormeku Engineering Company Ltd., Ghana) prior to starch extraction. The milled cassava was mixed with 25 L of water for crude starch extraction using a laboratory sieve of 250- μ m² mesh (DIN 4188, Prüf-sieb). The amount of starch obtained from each accession was expressed as a percentage "on dry weight basis" as shown in the equation below:

$$\% \text{ Starch yield (dry weight)} = \left(\frac{X}{Y} \right) \times 100$$

Where, X = dry weight of starch extracted; Y = fresh weight of peeled cassava tuberous roots.

Dry matter determination

A clean evaporating dish was oven dried at 105°C for 30 min to eliminate any trace of moisture and allowed to cool in a desiccator for 15 min and then weighed. 150 g of peeled cassava tuberous root chips were dried at 60°C for 72 h in an oven (Gallenkamp, United Kingdom) followed by cooling for 30 min in a desiccator. Each accession was replicated three times and the weight of each dried sample was expressed as a percentage of the fresh weight.

$$\% \text{ Dry Matter} = \left(\frac{\text{Weight of Fresh Sample (g)}}{\text{Weight of Dried Sample (g)}} \right) \times 100$$

Estimation of total starch

Total starch was estimated using a protocol described by Thayumanavan and Sadasivam (1984); and Njock et al. (1996). Cassava chips (0.1 to 0.5 g) were homogenized in hot ethanol

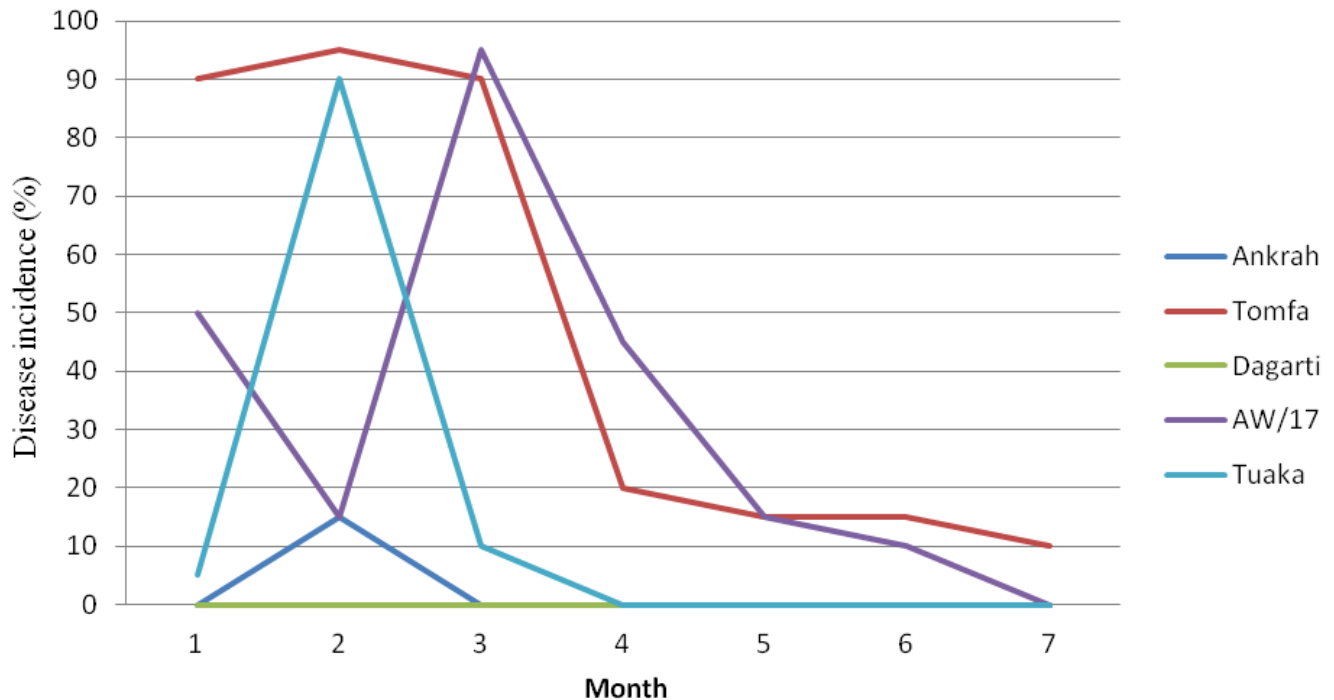


Figure 1. CMD symptom expression in five cassava accessions seven months after planting (MAP)

(80%) to remove sugars, centrifuged at 2500 rpm for 10 min and washed repeatedly with hot ethanol (80%) until no colour was observed with anthrone reagent. The residue was dried over a water bath before addition of 5.0 ml distilled water and 6.5 ml of 52% perchloric acid. The mixture was again centrifuged twice with 52% perchloric acid and 0.1 ml of the supernatant was pipetted and made up to 1 ml with distilled water. Standard glucose solutions were prepared at concentrations of 0.2, 0.4, 0.6, and 0.8%, and made up to 1 ml with water respectively. 4 ml of anthrone reagent was added to each standard solution tube, heated for 8 min in a boiling water bath and rapidly cooled. The intensity of green to dark green colour was read at 630 nm using a spectrophotometer (Shimadzu UV-1204, Australia). Glucose content in each sample was estimated using a standard graph and percent starch calculated based on a method described by Njock et al. (1996). The experiment was run in triplicates and the mean starch content for each accession was estimated.

Statistical analysis

Statistical analysis was performed using Minitab Statistical Software Version 15. Analysis of variance (ANOVA) was done to separate differences between means of treatments. Data on CMD score was subjected to square root transformation before analysis.

RESULTS AND DISCUSSION

Symptom severity and disease incidence

Three cassava accessions namely Tomfa, AW/17 and Tuaka showed visual symptoms of the mosaic virus disease one month after planting (Figure 1) while Ankrah

and Dagarti accessions showed no symptoms. Of the three accessions that showed typical CMD symptoms, Tomfa had the highest disease incidence (DI) of 95% followed by AW/17 (50%) and Tuaka (5%). CMD symptoms increased at two months after planting with all the accessions showing infected plants (Figure 2) except Dagarti, which remained symptomless throughout the study period. At infection peak, 95% of Tomfa and 90% of Tuaka plants displayed infection as indicated by symptom expression, while only 15% of Ankrah and AW/17 showed CMD symptoms suggesting a level of resistance or tolerance to CMD in both accessions. Almost all the accessions showed recovery from mosaic symptoms four months after planting and by the end of the seventh month, there was little or no symptom of mosaic disease suggesting recovery of infected plants. The recovery of CMD-infected plants over time has been reported (Fargette et al., 1996; Njock et al., 1996).

According to Gibson and Otim-Nape (1997), the recovery and reversion of CMD- infected shoots was enhanced during hot periods and delayed during cold periods of the year. The use of thermotherapy by hot-air treatment of cassava cuttings proved effective in eliminating ACMD from some East Africa cultivars Kaiser and Louie (1982). It appears viral multiplication is suppressed or inhibited in infected plant tissues at high temperatures (Acheremu, 2009) and with annual average temperatures between 26 to 28°C in the study area (MOFA, 2010); recovery of infected shoots after the seventh month was observed. The ISS_{AP} and ISS_{DP}



Figure 2. Accession Ankrah showing (a) a healthy looking plant and (b) a CMD infected plant

Table 1. Index of severity of symptoms on all plants (ISS_{AP}) and diseased plants (ISS_{DP}) in five cassava accessions recorded for seven months after planting.

MAP	Severity	Accession				
		Ankrah	Tomfa	Dagarti	AW/17	Tuaka
1	ISS_{AP}	1.00	2.95	1.00	1.55	1.05
	ISS_{DP}	0.00	3.05	0.00	2.10	2.00
2	ISS_{AP}	1.15	3.70	1.00	1.15	2.35
	ISS_{DP}	2.00	3.84	0.00	2.00	2.50
3	ISS_{AP}	1.00	2.40	1.00	1.10	2.20
	ISS_{DP}	0.00	2.55	0.00	2.00	2.26
4	ISS_{AP}	1.00	1.10	1.00	1.55	1.00
	ISS_{DP}	0.00	2.00	0.00	2.22	2.00
5	ISS_{AP}	1.00	1.15	1.00	1.00	1.15
	ISS_{DP}	0.00	2.00	0.00	0.00	2.00
6	ISS_{AP}	1.00	1.20	1.00	1.00	1.10
	ISS_{DP}	0.00	2.00	0.00	0.00	2.00
7	ISS_{AP}	1.00	1.15	1.00	1.00	1.00
	ISS_{DP}	0.00	2.00	0.00	0.00	0.00

ISS_{AP} , 1.00: means no symptom expressed; ISS_{DP} , 0.00: means no diseased plant recorded; MAP, months after planting.

followed a similar trend observed in disease incidence. Thus, ISS_{AP} and ISS_{DP} increased in the initial stages of shoot development and decreased after three months. The highest ISS_{AP} (3.70) and ISS_{DP} (3.84) were observed in Tomfa at two months after planting suggesting that this accession is highly susceptible to CMD (Table 1). Dagarti remained symptomless throughout the study period with no reduction in foliage indicating that it is highly tolerant to CMD. Thus, cultivation of Dagarti in areas where cassava leaves are used as vegetable in

dishes will be appropriate. Also, because most Ghanaian cassava varieties exhibit tolerance to CMD, it is important that molecular testing for example PCR be used to index planting materials prior to dissemination.

Molecular indexing

All the five accessions screened showed the presence of ACMV, while three accessions (Dagarti, Ankrah and AW/17) showed a dual infection of ACMV and EACMV

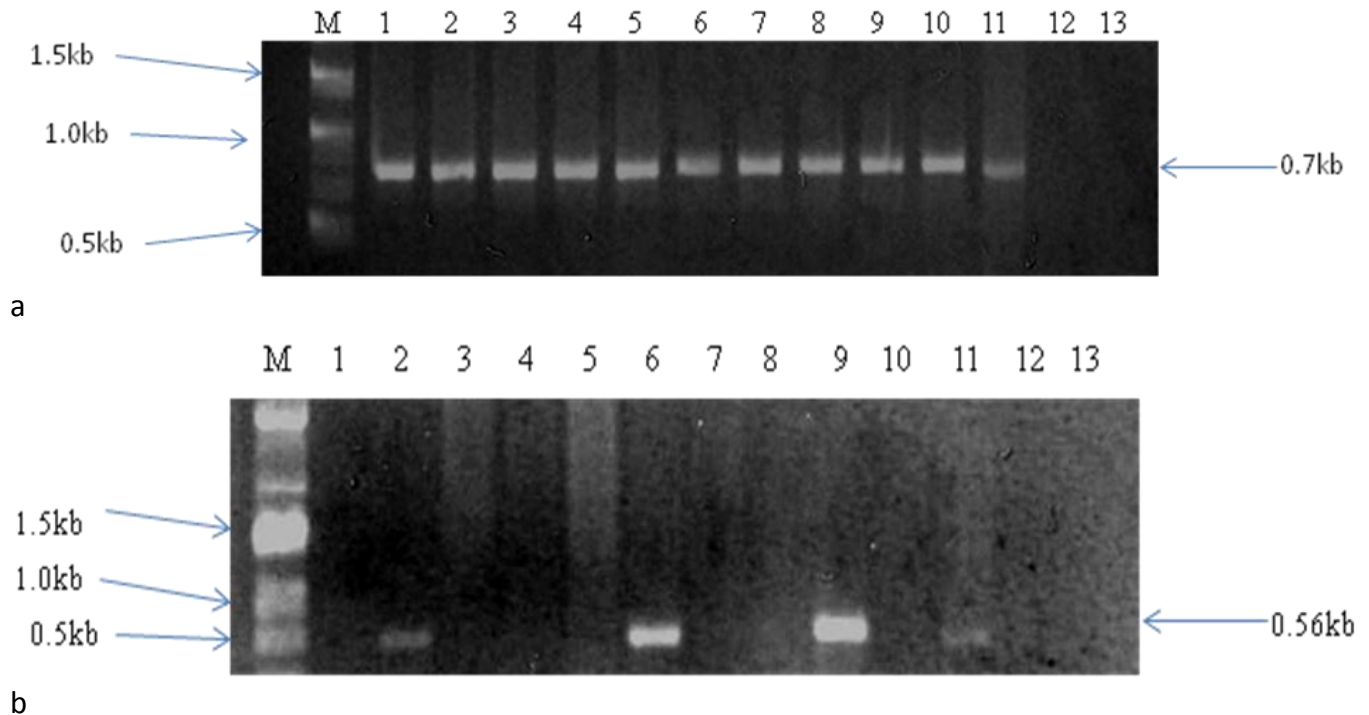


Figure 3. PCR amplification of cassava DNA using (a) JSP001/JSP002 and (b) EAB555 F/R primers for detection of ACMV and EACMV, respectively. M = 1 kb DNA ladder; 1 = Dagarti a; 2 = Dagarti b; 3 = Tuaka a; 4 = Tuaka a; 5 = Ankras a; 6 = Ankras b; 7 = Tomfa a; 8 = Tomfa b; 9 = AW 17a; 10 AW17 b; 11 positive control; 12 = negative control; 13 = water control.

(Figure 3). However, none of the accessions tested positive for the virulent Ugandan strain of EACMV. Until recently, EACMV and EACMV-Ug were only prevalent in East and Central African countries. The present observation confirms earlier reports by Offei et al. (1999) and Were et al. (2003) in which cassava leaves collected from Ghana were infected with both ACMV and EACMV but not with the Ugandan variant. The high rate of recombination of different geminiviruses co-infecting cassava (Patil and Fauquet, 2009) can result in the formation of virulent strains which reduce yield as observed in the Ugandan variant (EACMV-Ug) (Pita et al., 2001). Furthermore, the presence of both ACMV and EACMV in Ghana may be one of the contributing factors to low cassava yields in the country. The non-detection of EACMV-Ug may indicate the absence of the virus in Ghana.

Tuber yield

The mean number of tubers harvested was comparatively higher (7.41) in Ankras than the other accessions with Dagarti recording the lowest (5.33). However, there was no significant difference ($P \leq 0.05$) between yields in the two accessions (Table 2). There was a weak, non-significant ($P > 0.05$) positive correlation ($r = 0.2$) between CMD incidence and number of tubers produced. This indicates that the number of tubers produced is

genotypic and likely not affected by CMD infection. In Africa, most farmers are not worried about the incidence of CMD because in spite of the disease, cassava varieties still give appreciable yields. Moreover, the susceptible varieties sometimes possess farmer-preferred qualities or traits which promote their continued cultivation (Okorogri et al., 2010). There was a negative correlation ($r = -0.5$) between CMD and mean tuber yield in all five accessions suggesting that the higher the incidence of CMD, the lower the tuber yield. Earlier reports by Tumwesigye et al. (2006) showed a negative correlation between CMD incidence and tuber weight (yield) under natural disease infection conditions in several cassava genotypes. The damaging effects of CMD, that is, chlorosis and reduction in leaf size especially at the early and most susceptible stage of plant growth results in a reduction in photosynthetic activity thereby affecting partitioning of photoassimilates from the leaves to storage roots (Mariscal et al., 2002). Reduced tuberization and smaller yields due to reduced photosynthetic activity resulting from chlorosis caused by CMD have been reported (Thresh et al., 1997; Osiru et al., 1999).

Dry matter yield

Dry matter yield at 12 months after planting was significantly ($P \leq 0.05$) higher in Dagarti than the other acces-

Table 2. Effect of CMD incidence on fresh tuber weight (tuber yield) of five cassava accessions 12 months after planting (MAP).

Accession	Mean CMD incidence	Mean number of tubers	Mean tuber yield (kg / plant)
Dagarti	0.04 ± 0.00 ^a	5.33 ± 0.25 ^a	0.62 ± 0.09 ^b
Ankrah	1.06 ± 0.32 ^a	7.41 ± 0.15 ^a	1.03 ± 0.10 ^{ac}
Tuaka	1.60 ± 0.31 ^a	5.75 ± 0.29 ^a	0.43 ± 0.08 ^b
AW/17	31.29 ± 0.66 ^b	6.58 ± 0.18 ^a	0.76 ± 0.13 ^{bc}
Tomfa	39.26 ± 0.61 ^b	6.25 ± 0.29 ^a	0.08 ± 0.11 ^b

*Values in the same column followed by the same superscripts are not significantly different at $P \leq 0.05$ according to Tukey's pairwise comparisons; **Mean CMD incidence was estimated as a percentage of CMD diseased plants averaged over a 7-month period.

Table 3. Effect of CMD incidence on dry matter and starch content in five cassava accessions 12 months after planting (MAP).

Accession	Mean CMD incidence	Dry matter Content (%)	Starch yield (%)
Dagarti	0.04 ± 0.00 ^a	37.34 ± 0.31 ^a	65.90 ± 0.20 ^b
Ankrah	1.06 ± 0.32 ^a	34.70 ± 0.38 ^b	68.50 ± 0.23 ^a
Tuaka	1.60 ± 0.31 ^a	23.97 ± 0.43 ^d	64.01 ± 0.22 ^c
AW/17	31.29 ± 0.66 ^b	34.56 ± 0.77 ^b	60.10 ± 0.17 ^d
Tomfa	39.26 ± 0.61 ^b	29.20 ± 0.62 ^c	58.32 ± 0.27 ^e

Values in the same column followed by the same superscripts are not significantly different at $P \leq 0.05$ according to Tukey's pairwise comparisons.

sions. Even though there was a weak, non significant ($P > 0.05$) negative correlation ($r = -0.3$) between CMD incidence and dry matter yield, an increase in CMD incidence caused a decrease in tuber weight. This observation is expected since tuber weight or bulking involves dry matter accumulation and any factor which affects assimilate supply directly affects weight of tuberous roots produced (Mariscal et al., 2002). However, some genotypes are able to quickly recover from CMD symptoms with little impact on tuber bulking and dry matter production (Thresh and Cooter, 2005) as seen with Ankrah genotype. Thus, the accessions Dagarti and Ankrah, which had the lowest CMD incidence and severity produced the highest dry matter content (Table 3).

This high production of dry matter could also be genotype-influenced but the effect of CMD cannot be ignored since symptoms reach peak in the first three months after planting. The bulking of tuberous roots begin 30 days after planting reaching maximum levels between the third to sixth months before a gradual reduction in the rate (Howeler and Cadavid, 1983; Ekanayeke et al., (1997), hence the presence of the virus at the early stages (first three months) of growth reduces photosynthesis and hence yield (Pita et al., 2001). Thus, a high CMD incidence during the early stages of growth affects dry matter accumulation. The Dagarti accession did not phenotypically express any mosaic virus symptoms throughout the study period and this probably accounted for the high dry matter content recorded.

Starch yield

The yield of starch was significantly ($P \leq 0.05$) high in

Ankrah (68.50%) followed by Dagarti (65.90%) as shown in Table 3. Tomfa which had the highest CMD incidence also recorded the lowest starch yield. The most visible symptom of cassava mosaic disease is the reduction in leaf area and chlorosis resulting in a reduction in chlorophyll content ultimately reducing the photosynthetic capacity of the affected plants. (Calvert and Thresh, 2002). Cassava varieties with high dry matter content and starch yield are traits that make cassava suitable for use in the starch manufacturing as well as baking industries. Cassava dry matter and starch are also important in the acceptability of cassava as food and for dietary purposes (Hahn, 1989). This implies that both Ankrah and Dagarti accessions will be suitable for the wood and textile industries, where starch quantity is of prime importance. However, in the short term, cleaning of planting materials via thermotherapy and meristem culture can reduce the burden of CMD in cassava cultivars whilst the use of genetic transformation can rapidly help introgress improved virus resistance traits into both industry and farmer-preferred varieties. Starch yield was negatively correlated ($r = -0.4$) to CMD incidence in all five accessions.

Conclusion

The presence of mosaic virus disease in cassava is very pronounced in the first five months after planting. Even though all accessions showed recovery with time, the extent of recovery and ability to mask CMD symptoms was influenced by genotype. Independent of genotype, tuber and starch yield was negatively correlated to cassava mosaic disease incidence. This implies that a

high mosaic incidence particularly in the first three months of vegetative growth results in lower tuber and starch yields, thus, affecting calorific intake of many people who depend on cassava as a major staple especially in Sub-Saharan Africa. Furthermore, data on the susceptibility or tolerance of some local accessions to cassava mosaic disease can play a key role in the selection of accessions for cultivation in CMD endemic zones.

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