Association between accumulation of total cyanogens and progression of cassava mosaic disease in cassava (Manihot esculenta Crantz)

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Association between accumulation of total cyanogens and progression of cassava mosaic disease in cassava (*Manihot esculenta* Crantz)

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Abstract

Cyanogenic plants, such as cassava, exhibit genetic resistance to diseases and pests, which is associated with a general defence signalling mechanism that responds to stress situations. Cyanogens in cassava vary diversely with cultivar, stress, location, environment and plant age. Factors that induce plant stress often result in increased total cyanogens. Interestingly though, increased total cyanogens often associate with increased resistance to stress. This overlap between plant defence systems and plant stress is not coincidental. The infection of cassava with diseases and pests not only affects yields, but also influences the level of total cyanogens, which are presumed to be a part of the plant defence system. To qualify the extent to which total cyanogens are influenced by cassava mosaic disease (CMD), the effects of CMD status, cultivar resistance and plant vigour on total cyanogens accumulation in cassava at different growth stages of the crop was investigated. A total of 436 cassava plants were studied in the field and greenhouse, representing three different CMD status categories i.e., resistant, moderately susceptible and highly susceptible genotypes. Associated cyanogenic potential (CNp) levels were determined using standard protocols. CMD and CNp were shown to be highly associated (r = 0.90, P d" 0.001 for variety; Pd" 0.003 for CMD) suggesting a role of CNp in the defence mechanism against CMD infection. Notably, lower cyanogen levels occurred in vigorous highly susceptible cassava than in the non-vigorous highly susceptible (most stressed), suggesting a positive correlation between stress level and CNp content. The results of the greenhouse studies matched with those from the field further supporting the observed association between CMD and CNp (P d" 0.001), although, CNp levels were lower than in field trials (by 19.3 fold for roots and 6.3 fold for leaves) possibly due to temperature and nutrition effects. Future studies including understanding of the genetic link between CNp and CMD, interaction between vigour x variety x CMD status, effects of seasonality on CNp accumulation, temperature regimes and nutrition on CNp yield, together may further our knowledge and capacity to control CMD.

Key words: Association, progression, total cyanogens, *Euphorbiaceae*, linamarase, poisoning

Introduction

Cassava (*Manihot esculenta* Crantz) is a monoecious perennial woody shrub of the family *Euphorbiaceae*. It ranks fourth world wide as a source of dietary carbohydrate energy (720.1 x 10^{12} kJ day^{-1}) after rice, sugarcane and maize, sustaining over 600 million people (Mann, 1997; Baguma *et al*., 2003). An important feature of cassava is that it contains cyanogens, which release toxic hydrogen cyanide (HCN) upon degradation by the endogenous enzymes in a process known as cyanogenesis (Koch *et al.*, 1992; Bokanga, 1994). The resulting cyanogens can cause acute poisoning and various toxico-nutritional diseases of humans (Rosling and Tylleskär, 1995).

The interactive effects of environment, diseases and total cyanogens have long been known to modulate cyanogenesis, and co-occurrence of cyanogenic substances and specific catalytic enzymes (Anyanru and Sharma, 1984; El-Sharkawy, 1993; Brimer, 2000). For instance cyanogens were reported to confer defence in polymorphic species where cyanogenic and acyanogenic plants co-exist (Mahungu *et al.*, 1994). In separate studies, acyanogenic plants were observed to be more susceptible to herbivores than ones containing substantial amounts of the compound (Jones, 1966). By contrast to defence response, cassava suffers from yield losses (20 – 90%) due to a number of abiotic and biotic factors (Fauquet and Fargette, 1990) of which the devastating effects of cassava mosaic disease (CMD) are arguably responsible for greater reductions than any other pest or disease. It suffices to mention that, such occurrences allude to one pertinent fact that CMD might be involved in influencing cyanogenic potential (CNp) accumulation. To increase our understanding on the role of plant stress in cassava, the effect of CMD on yield and distribution of total cyanogens in different plant parts of a range of varieties was studied. The objectives of this study were to (i) determine the accumulation patterns of total cyanogens in the storage roots and leaves of cassava varieties with varying resistance levels to CMD, and (ii) establish the relationship between plant vigour and total cyanogens accumulation patterns in cassava plants of similar health status.

Materials and methods

Study location

The study was conducted at Namulonge Agricultural and Animal Production Research Institute (NAARI) during the 2002/2003 cropping season. NAARI is located at 0^0 32N, 32^0 37E, at 1150 m a.s.l., with an average temperature of 27^0C, 65% humidity, mean rainfall 1270 mm/annum and typified by clay gravelled loam soils.

Plant material and growth conditions

Three cassava varieties (Bao, Nase 3 and Tongolo) were evaluated. They were chosen because the varieties differ in their total cyanogen levels i.e. low cyanogenic, Bao (0 – 100 mg HCN/kg dry weight); medium cyanogenic, Nase 3 (TMS 30572) (100 – 300 mg HCN/kg dry weight); and highly cyanogenic, Tongolo (> 300 mg HCN/kg dry weight). In addition, the varieties represent a range of CMD classes i.e. CMD-resistant (Nase3) and highly susceptible landraces (Bao and Tongolo). The clean planting materials were used in the study and only whitefly-infected varieties were sampled and analysed for CNp. The effects of early, mid or late infection were considered by scoring and sampling at different growth cycles of the plant as described (this report). This was effected by assigning scores with colour codes tagged on cassava plants. The effects of confounding factors (biotic and abiotic) on total cyanogens were considered by conducting two trials-one in the greenhouse and the other in field. In the field trial, the varieties were assigned singly 100 plants per plot (10 m x 10 m) at 1.0 m x 1.0 m spacing in four replications in a randomised complete block (RCBD) split plot design. To obtain superior degree of precision for CMD response, the variety and CMD (healthy-score 1, moderately susceptible-score 2-3, highly susceptible-score 3-4) were assigned to the main and sub-plots (after infection) respectively. This was done because (i) the effect of variety had been determined in earlier studies (e.g. Bokanga, *et al,* 1994), (ii) to increase the chance of detecting the differences amongst the CMD categories, and (iii) for experimental boarder expediency to reduce the border effect of the variety. By contrast, the greenhouse design took the form of stem cuttings 6 - 9 cm long, placed two-thirds vertically in 1litre buckets. The varieties were assigned singly 25 per plot (5 m x 5 m) at 0.5 m x 0.5 m spacing in four replications as designed for the field experiment (this report). During this investigation, twenty viruliferous whiteflies were released per plant to inoculate the plant with CMD as described by Omongo (2003). In brief, the twenty whiteflies were collected by pooters from the field of highly infected cassava and placed onto the apex of 1-month old healthy cassava plants. The *B. tabaci* adults were given an inoculation access period of 48 h before being removed as described earlier (Colin *et al.,* 2004). The estimate percentage of infective individual adult *Bemisia tabaci* was not determined (in this study) but was assumed to be 10% (Colin *et al*., 2004). The conditions in the greenhouse were estimated at 25,000-30,000 lux, 23^0C and relative humidity (RH) 40-70%. Cassava storage roots and leaves were sampled for CMD severity changes and cyanogenic potential analyses starting at 4 months after planting (MAP) until 12 MAP.

CMD progression

The changes in CMD severity and their relation to changes in cyanogenic potential (CNp) were studied from 4 to 6 months after planting (MAP) as described elsewhere (Bokanga, *et al.,*1994; Colin *et al*, 2004). Data on CMD infection – stratified by infection mode- were collated by the established scoring system (Otim-Nape and Thresh, 1998). The data ratings included CMD symptom severity and incidence on a 5-point scale, damage by cassava bacterial blight (*Xanthomonas axonopoldis* pv. *Manihoti*), cassava green mite (*Mononychellus tanajoa*) and estimate of adult whitefly (*Bemisia tabaci*) populations recorded as described (Omongo, 2003).

Determination of cyanogenic potential and rating of cassava mosaic disease severity

Assays of cyanogenic potential (CNp) were made on samples prepared from cassava leaves and storage roots at 4, 6, 8, 10 and 12 MAP for each CMD rating categorized in three sections i.e. healthy (score 1), moderately susceptible (score 2) and highly susceptible (score 3-5) according to standard protocols (O'Brien *et al.,* 1991*,* Essers *et al.,* 1993 and Bokanga, *et al.,* 1994). Resulting CNp data were tallied with corresponding CMD severity ratings (based on shoot symptom severity). CNp content and CMD severity data were subjected to analysis of variance using GENSTAT (Lawes Agricultural Trust 1998). Significant changes and relations between CMD and CNp were derived using ANOVA. Where "F"statistics indicated significance, the means were separated using Fisher's protective Least Significant differences (P 0.05) and compared using the t-test. Significant differences were shown using graphical analyses (Microsoft ®2000) with errors bars inserted.

Table 1: The mean total cyanogens (mgHCN/kg dry weight) in cassava varieties of different CMD infection levels grown at Namulonge, March 2002 compared by t-test at the 95% significance level.

Healthy status	Bao	Nase 3	Tongolo	Overall means
Healthy	85.3^{ax}	135.0^{ay}	569.0^{az}	263.1^{h}
Moderately susceptible	137.8^{bx}	189.8^{ax}	651.7^{by}	326.4^{i}
Highly susceptible	193.7^{cx}	238.4^{bx}	852.7^{bcy}	428.3^{j}
Overall means	138.9^{d}	187.7^{e}	691.1^{f}	

The means followed by the same letter are not significantly different from each other. The means followed by different letters (a, b, c and x, y for between healthy status and varieties respectively) are significantly different from each other by t-test (0.001). Overall means followed by different letters (d,e,f,h,I,j) are significantly different from each other by t-test (0.001)

Healthy (score 1) = No shoot symptoms
Moderately susceptible (score 2 – 3) = mild shoot symptoms
Highly susceptible (score 4 – 5) = severe shoot symptoms

Results and discussion

Both field and greenhouse experiments demonstrated that fluctuations in levels of cyanogenic potential (CNp) associate strongly with CMD symptom severity in different cassava cultivars. Moreover CMD status was associated strongly with increasing CNp content (Fig.1 and Table1). The patterns of leaf and root CNp accumulation differed significantly between variety and CMD category (P 0.001 for variety; P 0.003 for CMD) suggesting a combined effect of variety and CMD on the ultimate CNp levels. Consistently, the highest and lowest CNp levels in both Tongolo and Bao were recorded in CMD-susceptible and healthy cassava, respectively (Fig. 1).

Overall, CNp accumulation decreased with age for all variety and its initial CMD status (at 4 Months after planting) (Fig. 2). Most notably, CNp level declined to its lowest point by 8 MAP and changed little thereafter. Nonetheless, significant differences in CNp accumulation were detected amongst healthy and highly susceptible cultivars throughout, but not between healthy and moderately susceptible at 6 and 8 MAP in leaves. However, a comparison of susceptible and healthy cassava showed a rapid accumulation of CNp in roots of susceptible cassava throughout the entire growth period than in healthy cassava (Fig. 2). Least and highest cyanogens accumulation was recorded in healthy and highly susceptible varieties, respectively (Fig.

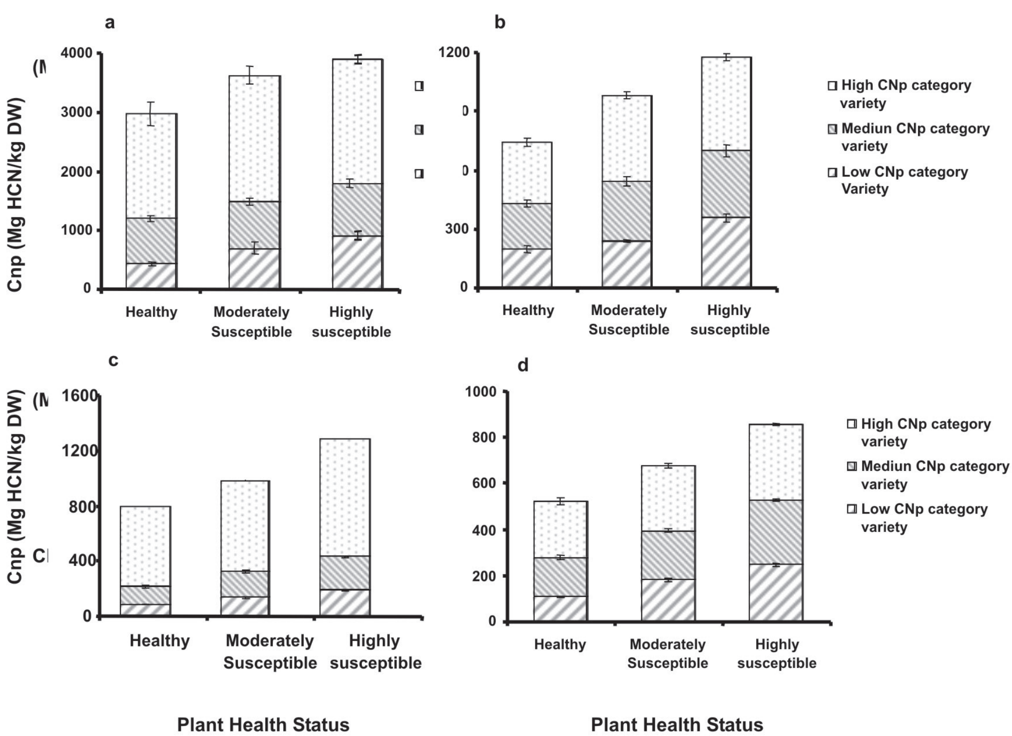


Figure 1. Influence of CMD on total cyanogens in cassava.
(a) and **(b)** Plant healthy status levels and leaf CNp for cassava grown under field **(a)** and greenhouse **(b)** conditions.
(c) and **(d)** Plant healthy status levels and root CNp for cassava grown under field **(c)** and greenhouse **(d)** conditions.

1), suggesting that high and low infections in inherently low and high cyanogens varieties associate with a yet undescribed CNp-CMD-dependent resistance mechanism. It is notable that the generalities of this data single out for the first time that CMD increases CNp. This is consistent with previous work, in which CNp strongly correlated with stress factors (Fry and Myers, 1981; Anyanru and Sharma, 1984, CIAT, 1991 and El-Sharkawy, 1993), thus, revealing that both genetic resistance and abiotic factors might be linked to CNp and general defense signalling mechanism in cassava.

CMD x variety dependent CNp accumulation in storage roots

To gain insight into the occurrence of CMD-variety dependent CNp accumulation, the CMD x varietal changes in CNp accumulation were assessed separately and or in combination in storage roots. CMD, variety and CMD x variety effects were highly significant (95% Confidence Interval for mean: 249.4, 429.2; $P<0.001$) suggesting that both individual and pooled factors influence CNp content. Together, it was found that CNp accumulation strongly depended on variety and CMD status singly or combined, and varied with crop age (Fig. 1). The abundant cyanogen content of the two highly CMD susceptible varieties compared to the resistant cultivar suggest that genetic resistance might be linked to CNp as well as general defence signalling mechanisms in cassava. Regardless of its implication, it is known that the cyanogenic glucosides are synthesized in the leaves and petioles (Bediako *et al*., 1981), mainly in response to damage (Kakes 1990 and 1991), stress (Anyanru and Sharma, 1984), or as a consequence of complex defence signalling pathways (Hruska, 1988; Jones, 1988; Kakes, 1990, 1991). Consequently, the present study is consistent with the view that CNp production might be more augmented in response to danger, in this case CMD infection. It suffices to mention that, whereas this study demonstrated that CMD x variety interaction affects CNp yield, it did also underwrite the separate contribution of variety and CMD on CNp accumulation. In contrast, the study showed convincingly that CMD infection does not affect the overall pattern of CNp accumulation in a given variety but rather alters the actual yield.

CMD x CNp association in cassava leaves

As in storage roots, the patterns of cyanogen concentration in the leaves were similar but substantially greater (Fig. 1). Most notably, CNp accumulation differed significantly amongst CMD levels and varieties (95% P 0.001). However, there was no significant interaction effect for CMD and variety (P 0.324). Counts of whiteflies on the varieties

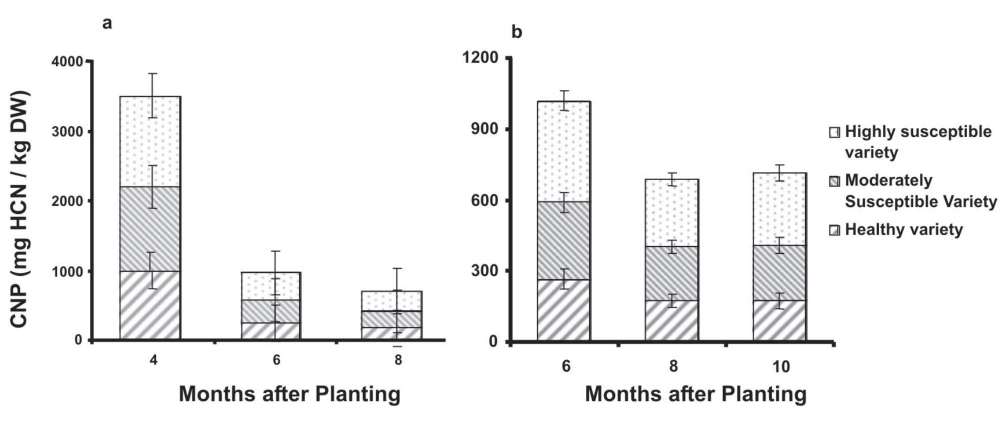
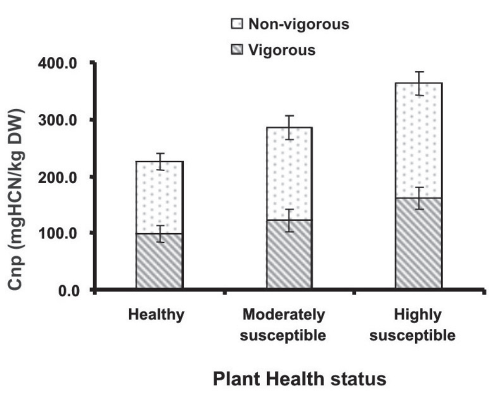


Figure 2. Influence of CMD on the pattern of leaf total cyanogens (**a**) and root total cyanogens (**b**) at various growth cycles of cassava.

(not presented) suggest that their feeding behaviour might account for leaf cyanogens profiles in addition to CMD infection. Variety Bao was the most preferred by whiteflies and concomitantly had the highest increase in cyanogens in the leaf after they were released and allowed to feed as compared to Tongolo and Nase 3 before feeding. The result supported the report of Omongo (2003), that the preferential feeding on cassava by the virus vector, *B. tabaci*, is related to the phloem content together with morphological and topographical differences in the leaf structure. However, as shown from the greenhouse CNp significant studies ($P < 0.001$) and earlier studies (El-Sharkawy, 1993; Mahungu *et al*., 1994 and Fry and Evans, 1997), the high cyanogens in varieties Bao and Tongolo could have arisen from a triggered response by both the intense feeding activity of the whiteflies, CMD infection and high incidence (not presented). Moreover, high whitefly populations and CMD incidence were consistently recorded in the non-vigorous Tongolo. It has also been reported elsewhere that the impact of heavy infestation of *B. tabaci* could apply equally on both CMD susceptible and healthy (not opposites) varieties in terms of damage by reduction in plant vigour (Legg *et al*., 2003) and ultimately reduction in crop yield (Byrne and Miller., 1990). Hence, there is a possibility from this study that the damage caused to non-vigorous varieties induced stress and resulted in high cyanogens accumulation. The high incidence on high cyanogens non-vigorous Tongolo could also be explained by favourable constituents essential for whitefly growth and reproduction such as amino acids, asparagines, glutamine, tryptophan and tyrosine as reported elsewhere (Colvin *et al*., 1999).

Analysis of similar data obtained from plants grown under greenhouse conditions showed comparable CNp accumulation pattern (P 0.001 for leaf; P 0.001 for root). However, the CNp yield *per se* was considerably depressed compared to equivalent field grown plants by 19.3 (storage roots) and 6.3 (leaves) fold. These data suggest that temperature and nutrition might be essential determinants of CNp yield in cassava. In this control experiment, it could not be concluded whether the accumulation of CNp in healthy and moderately CMD susceptible varieties is directly or indirectly caused by CMD changes in storage roots and leaves of cassava since greenhouse conditions failed to manifest obvious differences between the latter two CMD categories. However, within the limits of these data it can be speculated that CMD status associates with CNp accumulation pattern.

Figure 3. Total cyanogens accumulation amongst infected and less infected vigorous and non-vigorous cassava but of similar CMD status



Variations in cyanogens level in both infected vigorous and infected less vigorous cassava, but of similar CMD status

To compare the association of total cyanogens and the nature of plant vigour, the accumulation of total cyanogens was monitored in health vigorous and less vigorous, and infected plants of similar CMD status that were vigorous or less vigorous (Fig. 3). Despite the striking CMD status in both groups of plants, lower cyanogens contents accumulated in the highly susceptible vigorous cassava than in the less highly susceptible vigorous ones (95% P 0.013 for CMD; P 0.011 for nature of vigour). Similar results were observed amongst healthy plants and those of intermediate CMD status in the two vigour categories. Additionally, there was no significant difference by CMD and nature of vigour interactions (P 0.995). These results showed convincingly the strong association between cyanogens yield and lack of vigour. Conceivably, in the first case, in vigorous rapidly growing plants, there is greater assimilation of nitrogenous metabolites for growth rather than as a cyanogen reservoir. Second, the dramatic increase in CNp observed in non-vigorous plants strongly suggests enhanced recruitment of cyanogenic toxins to evade stress. While these hypotheses present exciting possibilities, they do not exclude modulation of cyanogens level by yet unknown naturally occurring mechanisms rather than those externally conditioned.

In conclusion, these results suggest that variety, CMD status, and vigour singly or combined, regulate the accumulation patterns of CNp in both the storage roots and leaves of cassava. While results from this study evidently support the hypothesised relationship between CMD status and CNp, that CMD might influence the production of CNp, the CNp accumulation profiles differ considerably. Overall, the data presented here indicate 1) progression in CMD status associates strongly with a positive increase in CNp yield, 2) changes in CNp accumulation correlate strongly with variety and CMD status singly and/or combined implying that genetic resistance might be linked to CNp potential since substantially higher levels of CNp were detected in CMD-susceptible than CMD-resistant cassava varieties, 3) whereas, CMD status has been shown to increase CNp yield, its presence or absence does not affect the overall CNp accumulation profile, 4) despite the striking CMD symptoms in both vigorous and non-vigorous plants, substantially lower cyanogen levels accumulated in vigorous than in non-vigorous plants. Similar results were observed amongst health and intermediary CMD status plants in the two vigour categories, thus emphasising the strong association between CNp yield and vigour, 5) as in the storage root, the patterns of cyanogens accumulation in the leaves were similar but contents were substantially greater. CNp accumulation in the leaves is likely to be associated with both selective feeding behaviour of the whitefly and CMD infection, and, 6) corroboration of field and greenhouse data demonstrated that temperature and nutritional functions are likely to be essential determinants of CNp yield in cassava. Further studies on the genetic link between CNp and CMD stress, the interaction between plant vigour x variety and CMD status in different agro-ecologies, and the effects of temperature and nutrition on CNp yield will enhance the knowledge and implications of stress factors.

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