academicJournals

Vol. 7(2), pp. 12-22, April 2015
DOI: 10.5897/IJPPB2015.0227
Article Number: 48E45A552094
ISSN 2006- 9871 © 2015 Academic Journals

http://www.academicjournals.org/IJPPB

International Journal of Plant Physiology and Biochemistry

Full Length Research Paper

Effect of cassava brown streak disease (CBSD) on cassava (*Manihot esculenta* Crantz) root storage components, starch quantities and starch quality properties

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Received 13 February, 2015; Accepted 19 March, 2015

Effect of cassava brown streak disease on cassava root storage components were studied on four Ugandan varieties with varying levels of tolerance. Significant differences (P<0.05) were observed with reductions of 30% in amylose content and 50% in amylopectin content of diseased compared to healthy plots. Average dry matter content of diseased plots was 25% higher as much as starch yield and starch content reduced by 40 and 15% respectively in diseased plots compared to healthy plots. Susceptible varieties had lower protein and higher cyanide contents in diseased state compared to tolerant varieties. On pasting, mixed reactions were observed but importantly there were significant differences (P<0.05) in the starch pasting properties of starch from diseased compared to healthy plots. Plants with similar reactions to viral attack at the phenotypic level had different reactions when the levels of particular metabolite components (especially cyanide and starch constituents) were quantified. The results point to hijacking of plant carbohydrate and nitrogen metabolic processes for viral metabolic gains. In turn, this affects the use of cassava for food and other applications but also points to possible use of metabolite based selections for tolerant varieties rather than mere root and stem phenotypic observations.

Key words: Brown streak disease, Cassava, metabolism, starch, plant virus.

INTRODUCTION

Cassava is vulnerable to a broad range of diseases caused by viruses including the cassava brown streak viruses, a range of cassava mosaic viruses and the less known and less potent viral strains across the tropical cassava growing regions (Alabi et al., 2011). In Uganda, the most potent viruses are the cassava brown streak virus groups (Alicai et al., 2007, Odpio et al., 2013), a host of cassava mosaic viruses (Sserubombwe et al., 2008) and the less known, uncharacterized Kumi virus A and B (Alabi et al., 2011). Among them, cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) viruses are the most severe and widespread, limiting production of the crop in sub-Saharan Africa.

Cassava viruses especially the cassava brown streak

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viruses induce several morphological modifications in the root and are thus thought to have significant effects on root storage components. They produce a variety of foliar symptoms that include browning, early leaf senescence, mosaic, mottling, misshapen and twisted leaflets, and an overall reduction in size of leaves and plants (Alicai et al., 2007). The symptoms and accompanying cellular modifycations depend on whether cassava is infected with a single virus, or if there is a concurrent infection of two or more viruses resulting in synergistic interactions (Ogwoket al., 2012,).

There are big differences between cassava varieties in the type, extent and severity of the symptoms caused by cassava viruses where tolerant varieties express much less severe symptoms than susceptible ones, especially during the late stage of crop growth when tolerant varieties may even become symptomless (Calvert and Thresh, 2002). Symptom expression is also influenced by environmental factors and leaves produced during hot weather tend to be affected less than those produced at other times. Moreover, virulent strains cause more severe symptoms than avirulent ones and have greater effects on growth and yield. Such a complex puzzle of symptoms makes it difficult to ascertain disease severity and hence easily determine the extent of damage to the crop.

Much as there is no evidence of consistent differences between symptoms caused by the different cassava viruses, dual infection with two different viruses causes more severe symptoms than either virus alone, as reported in studies in Uganda and Cameroon (Ogwok et al., 2010; Fondong et al., 2000). For cassava brown streak disease, the noticeable symptoms occur on leaves with varying patterns of chlorosis and can be used to distinguish at least two types of CBSV isolates (Mbanzibwa et al., 2009). Leaf chlorosis appears in a feathery pattern, first along the margins of the secondary veins, later affecting tertiary veins and may develop into chlorotic blotches. Alternatively, the chlorosis may not be associated with the veins but appear in near circular patches between the main veins. There is considerable variation in foliar symptoms expression depending on variety, growing conditions, age of the plant, and the virus isolate involved in causing the symptoms (Ogwok et al., 2010). Some cultivars show marked foliar symptoms but without or delayed root symptoms and vice versa. With such complexity in system identification, biochemical phenotyping is required to specifically understand symptom diversity in the root and the leaves among cassava viruses and resultant effects on plant yield components which in effect affects the farmers that derive their livelihoods from cassava.

In addition, the observed symptoms are usually due to systemic viral infection that result into necrotic lesions, indicative of structural changes in the chloroplasts, altered carbon metabolism, and the accumulation of starch grains as has been observed in a number of plant species (Goodman et al., 1986). Chlorophyll get reduced in diseased plants compared to healthy plants due to either inhibition of chlorophyll synthesis or destruction of chloroplasts (Goodman et al., 1986) which may result into observed yellowing in cassava plants. The changes that occur hence forth affects storage root properties in the host plants by influencing sugar transport, carbohydrate levels and the amounts of the various sugars either in the phloem (Shalitin and Wolf, 2000) or in the storage organs (Tecsi et al., 1996). This also affects photosynthetic metabolism by reducing it significantly (Goodman et al., 1986) while increasing the net respiratory rate (Fraser, 1987). In particular, downstream effects of viral infection resulting from altered metabolism have been observed as changes in total reducing sugars content where the diseased plants tend to have high available metabolic sugar contents. This has been attributed to the need for use of carbon and carbohydrate sources for protein synthesis and production of abnormal proteins used for viral replication (Goncalcaves et al., 2005) but may be related to lack of chlorophyll and related pigments for carbon dioxide fixation (Handford and Carr, 2007) hence reductions in total starch contents (Singh and Shukla, 2009). Other studies have also shown that an increase in reducing sugars and a reduction in starch content may be due to viral induced higher starch hydrolase and lower ADP-Glc pyrophosphorylase activities (Tecsi et al., 1994) that results into the inhibition of starch accumulation and/increased starch degradation. Thus, from the above, sugar compositions change with viral attack from complex sugars to derivatives of complex sugars representing hydrolytic pathways.

Viruses can cause significant adjustments in short term photosynthetic storage and export (Olensiki et al., 1995) which in turn affects the accumulation of secondary metabolites after viral attack, as an important plant defense factor. The secondary metabolites such as cyanide activate the defensive signals allowing the induction of specific resistance mechanisms by the plant. Relatedly, nitrogen content increases in diseased compared to healthy plants due to production of less structural protein and nitrogen sources as the virus reverts the plant system to allow for its replication and multiplication. Such proteins are of no importance to the plant but occur mostly as physiological proteins (Selman and Grant, 2008).

From the above, it is apparent that an alteration in plant metabolism results into visible phenotypic and biochemical differences between the diseased and healthy plants. Such an alteration may directly influence the susceptibility of plants to viral attack and may serve to explain the diversity of symptoms presented after viral attack. Thus in this study, such alterations have been profiled at a macro level to explain the changes in the plants' main carbohydrate and nitrogen metabolism. This will be key in understanding the processes involved in viral infection and establishment of the virus within the plant and provide suggestive strategies for managing

cassava brown streak disease. It will also provide inferences on the apparent measure of susceptibility and/or tolerance based on biochemical manifestations rather than visual inferences.

MATERIALS AND METHODS

Plant material used

Four varieties of cassava were selected on the basis of their response to cassava brown streak disease and earlier observations on the level of tolerance to the disease (Ogwok et al., 2010). The varieties included the highly tolerant variety NASE 14 and the moderately tolerant variety TME 14. In addition, the susceptible varieties included the highly susceptible TME 204 and the moderately susceptible I/92/0067 (Plate 1). The varieties were established in a randomized complete block design (RCBD) trial involving both healthy and diseased plots for each of the varieties replicated four times in a low disease pressure location of Kayunga which was suitable for this experiment since the spread of the disease between the diseased and healthy plots was low. In addition, healthy plants maintained a healthy state for a long time in their growing cycle in this location compared to areas with high disease pressure. At 10 months after planting, the cassava was harvested and roots selected from each of the plots for further analysis. The selected roots from the diseased plots included a collection of roots while for lignin determination, roots with different disease scores (score 1-5) were considered. Score 1 (one) roots were considered healthy and with no visible root CBSD symptoms while score 2-5 were diseased roots with different root scores. For other measurements, at least two roots were selected from each of the selected five-seven plants in each plot and prepared for dry matter content determination and starch extraction by peeling and washing to remove dirt and any other debris.

Determination of dry matter content

Cassava storage root dry matter content (DM) was determined within 8-12 hafter harvest to avoid post-harvest physiological deterioration or moisture loss of the root using the method by Benesi (2005). Roots were randomly selected from each plot. The mid sections of selected roots were cut into thin slices using a knife, mixed thoroughly and a triplicate of 200 g samples (X_1) were dried at 105°C for 24 h. After removal from the oven, samples were weighed immediately (X_2). Dry matter content as a percentage (DM %) was calculated as follows:

$$\mathrm{DM}(\%) = 100 * \frac{\mathrm{X2}}{\mathrm{X1}}$$

Starch extraction and determination of starch yield

Cassava starch extraction was carried out using a method described by Benesi (2005) and modified according to Nuwamanya et al. (2010). Five hundred grams (500 g) of the fresh tuberous cassava roots were washed, peeled, and homogenized with 500-700 mL of 1 M NaCl (BDH) to aid the release of starch from the solution using a Waring blender. The mixture was stirred with a stirring rod for about 5 min and filtered using a triple cheese (muslin) cloth. The filtrate was allowed to stand for 1 h to facilitate starch sedimentation and the top liquid was decanted and discarded. 200 ml of distilled water was added followed by centrifugation at 3,000 *g* for 10 min. The starch was air-dried on aluminum pans at room temperature for 24 - 36 h and stored in plastic air tight containers at room temperature. The extracted starch from each of the plots for a particular variety was bulked before analyses. Starch yield (SY) was determined as a percentage of the extracted starch (ES) in grams from each plant in the plot to the total amount of fresh root (FR) in grams used for extraction using the equation below:

$$\mathrm{SY}(\%) = 100\mathrm{x}\frac{\mathrm{ES}}{\mathrm{FR}}$$

Determination of pH

The pH was determined using pH meter (UltraBasic, Denver Instruments Model UB10) equipment with a glass electrode by dissolving 10 g of the starch sample in 100 mL sterile distilled water. The mixture was thoroughly mixed to allow for improved dissolution of starch and any other components. The pH of the resulting solution was then determined in comparison to the pH of the processing water.

Determination of starch content and reducing sugars

The starch content was determined using a Megazyme total starch assay kit based on the AOAC method 996.11 by enzymatic hydrolysis of starch (0.1 g) using amylase/amyloglucosidases and quantification of glucose using glucose oxidase/peroxidase reagent. The reducing sugar content of the extracted starch samples were determined by dissolving 0.5 g of the starch powder in hot 95% ethanol for initial extraction. Reducing sugars extracted into the ethanol where then subsequently quantified using the Dubois et al. (1956) method of reducing sugar quantification.

Determination of total protein content and cyanogenic potential

Total protein determination was carried out using the Bradford method (Bradford, 1976) with adaptations to cassava starch by dissolving the samples in distilled water at 50°C. All reagents used were supplied by BDH laboratories. The cyanogenic potential was also determined using fresh samples by the method of Bradbury et al. (1994).

Determination of lignin content

Lignin content was determined according to Morrison et al. (1995) with modification for cassava. Cassava roots were ground into flour with particles of mess sieve size 40 as the extractive-free biomass sample. From this sample, the moisture content was determined using the oven method. 0.2 g oven dried samples were weighed in digestion tubes (50 ml falcon tubes). 1.5 mL of sulfuric acid were added to this sample and the uniform mixture was generated by stirring. The mixture was placed in a water bath at 30°C for 1 hafter which 42 ml of deionized water containing 3% sulfuric acid was added. The resultant mixture was placed in an autoclave set at 121°C for 1 h after which it was taken out and cooled in iced water bath. The mixture was then filtered with glass fiber into 50 ml beakers followed by re-filtration using double layered filter paper. The filter paper was then washed and dried in an oven at 105° C. The remaining solid was weighed and determined as Klason lignin. The amount of lignin was presented as the percentage of the total weight of the flour sample analyzed.

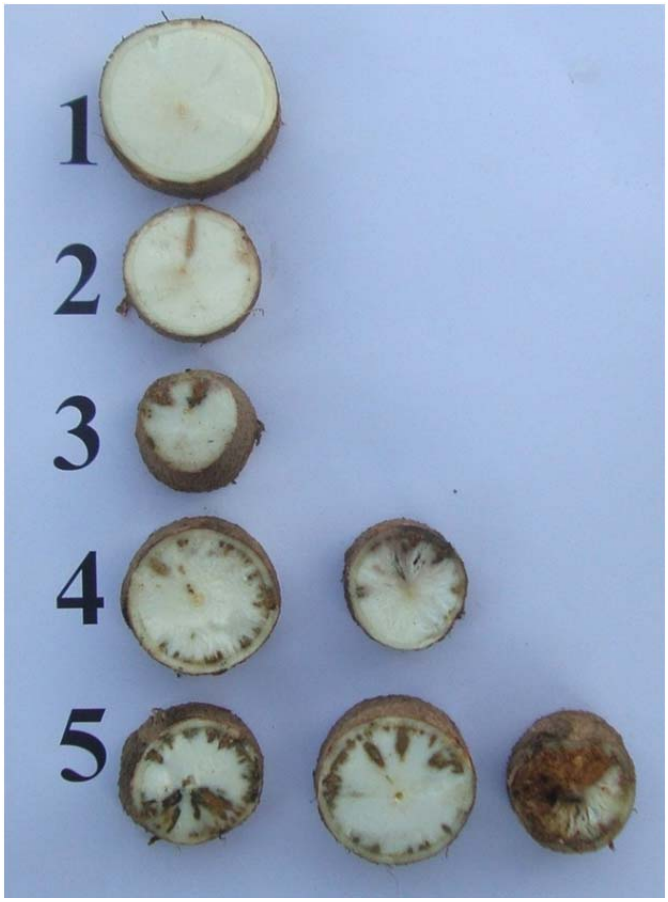

Plate 1. A pictorial representation of cassava roots with varying CBSD root scores depending on the observed symptoms. Score 1 represents asymptomatic roots from healthy and tolerant variety MH96/4271 while score 5 represents a diseased root with varying symptom expressions from variety TME 204.

Determination of amylose/amylopectin content

Starch (1 g) was dispersed into ethanol and then gelatinized using 0.1 M sodium hydroxide in a 5 mL solution. An aliquot (1.0 mL) was then obtained from the gelatinized solution and treated with an equal volume of citric acid (0.1 M). This was followed by addition of 3.5 mL of water and 0.5 mL of 10% iodine/KI solution. The absorbance of the resultant stained solution was then read at 620 nm to determine the concentration of amylose and then re-read at 680 nm to determine the apparent concentration of amylopectin. The ratio of the absorbance obtained at the different wavelengths was used to calculate the amylose/amylopectin ratios (Nuwamanya et al., 2009).

Determination of starch pasting properties

Cassava starch samples were milled and screened through a 0.5 mm sieve. To produce slurries, 3 g of the milled sample was weighed into an RVA canister. A volume of equal to 25 mL of distilled water minus the moisture present in the sample was added to the RVA canister. The RVA (RVA-4500, Perten Instruments, Australia) equipped with Thermocline software version 3 for Windows was held constant at 50° C, and mixing speed was set at 960 rpm for 10 sec followed by 14 minand 50 s at 160 rpm. Viscosity was recorded every 4 s, and the final viscosity was noted at the end of 15 min.

RESULTS AND DISCUSSION

There were significant differences for DM between the diseased and healthy plants for each variety ($p < 0.05$) with an average 4% increment in the DM for diseased plots compared to healthy plots (Figure 1), which can be attributed to accumulation of lignified tissues presented as brown necrosis within the root (Alicai et al., 2007). No significant differences ($p<0.05$) were observed between the tolerant varieties (TME 14, and MM96/4271) and the more susceptible varieties (I/92/0067 and TME 204) for each of the treatments used in terms of the DM, much as significant differences ($p<0.05$) were observed between the varieties tested. The specific variety differences in accumulation of root based “impurities” as DM may point to differences in the effect of the virus on plant photochemistry and assimilate movement as suggested by Sajnan et al., (2007).

The amount of pure starch produced per 100 g of fresh roots was high among the healthy plots compared to the diseased plots (Figure 2). Clear significant differences were observed among the treatments and among the varieties for starch yield with between 55-65% in starch reductions observed in the diseased treatments. This was expected since on viral attack, starch deposition in plant storage organs is compromised (Watson and Watson, 2008). Among the healthy plants, high starch yield was observed for TME 14 at 25% while low starch yield was observed for I/92/0067 at 21.8%. These differences point to inherent yield differences among these varieties with TME 14 having higher yield. On the other hand, differences for starch yield among diseased plots were also observed with tolerant variety MM96/4271 having high starch yields compared to the susceptible varieties (Figure 2). The low starch yield for I/92/0067 was consistent among the healthy and diseased plots much as it was highly diminished among the diseased plots. The reduction in starch yield observed in diseased plots can be attributed to reduction in photosynthetic starch production (Handford and Carr, 2000) as leaf morphology is affected by Cassava Brown Streak Virus (CBSV) attack. This is because CBSD leaf symptoms are characterized by leaf browning (Ogwok et al., 2010) hence possible chlorophyll losses and an alteration in the photosystems architecture that reduces the photosynthetic potential resulting into poor source strength. In addition, low starch quantities in the root may be due to effects of the virus on sink strength that arise from viral movement proteins that operate along the sieve elements affecting phloem loading and translocation as suggested by (Goodman et al., 1987). This alone can affect the type and amount of loaded photo assimilates which in turn affects what reaches the sink (Handford and Carr, 2000) and hence reduces on the total storable carbohydrate

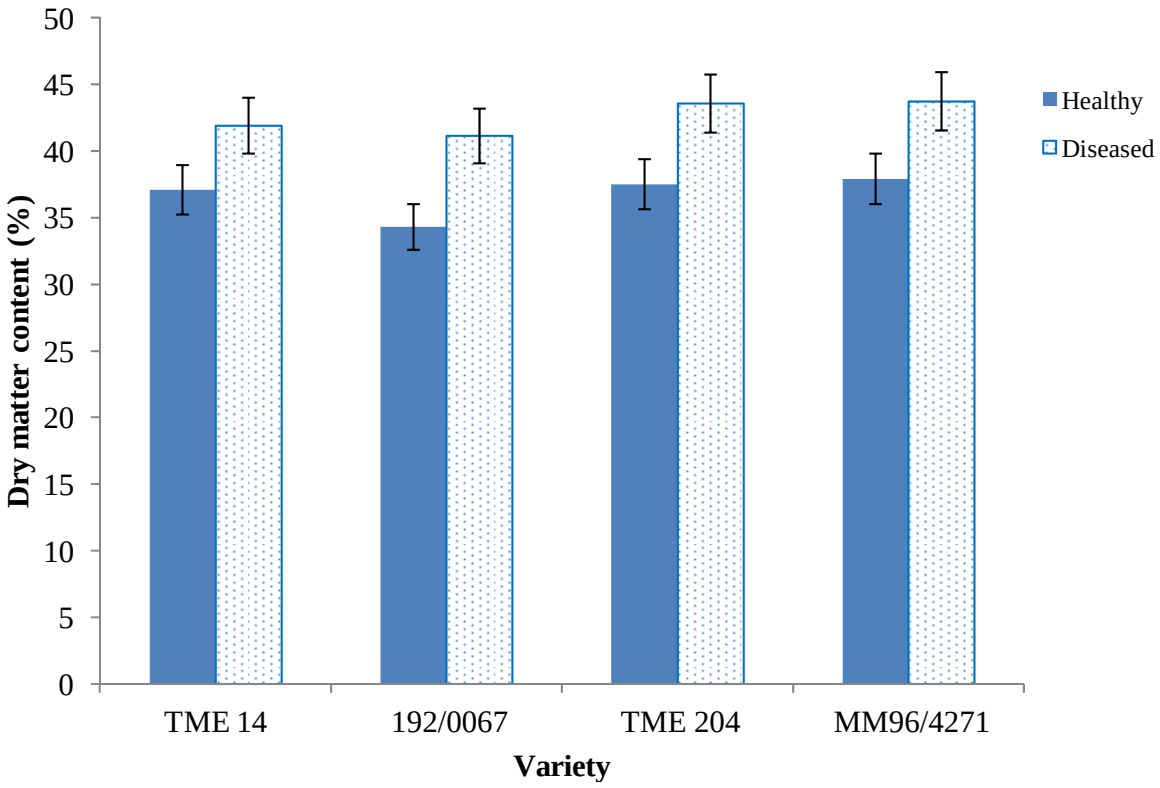

Figure 1. Average dry matter content for the different test varieties in both diseased and healthy plots.

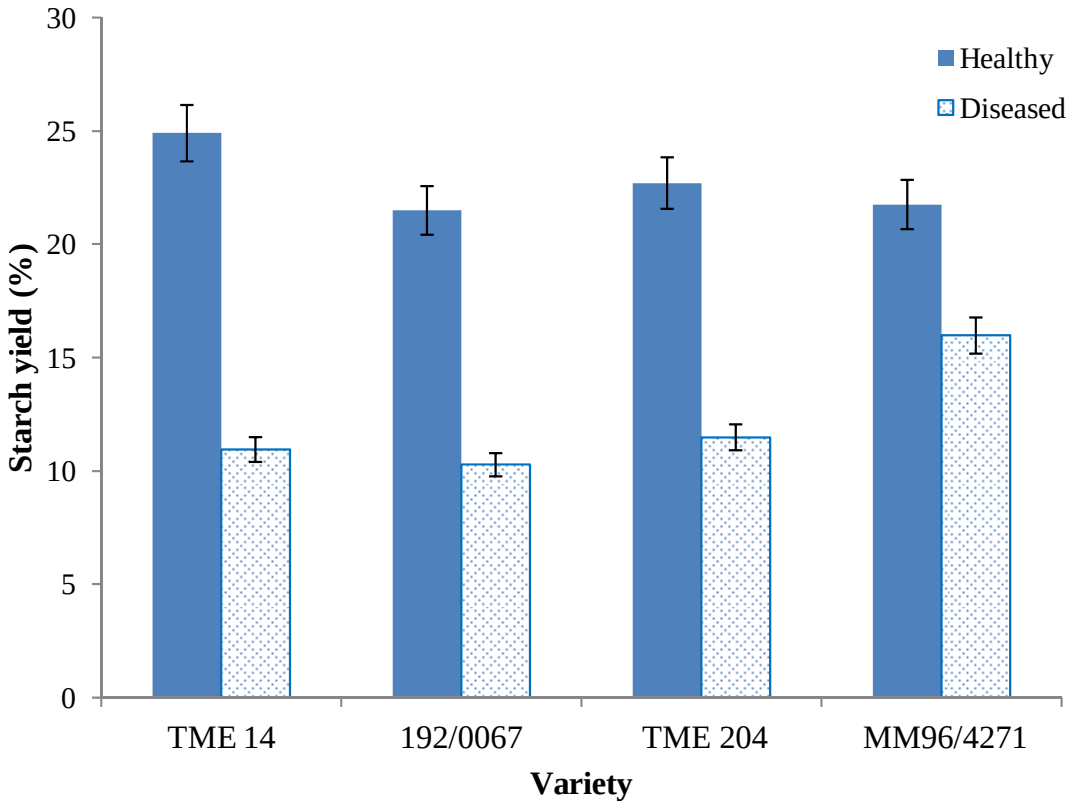

Figure 2. Starch yield variations from the different varieties in both diseased and healthy plots.

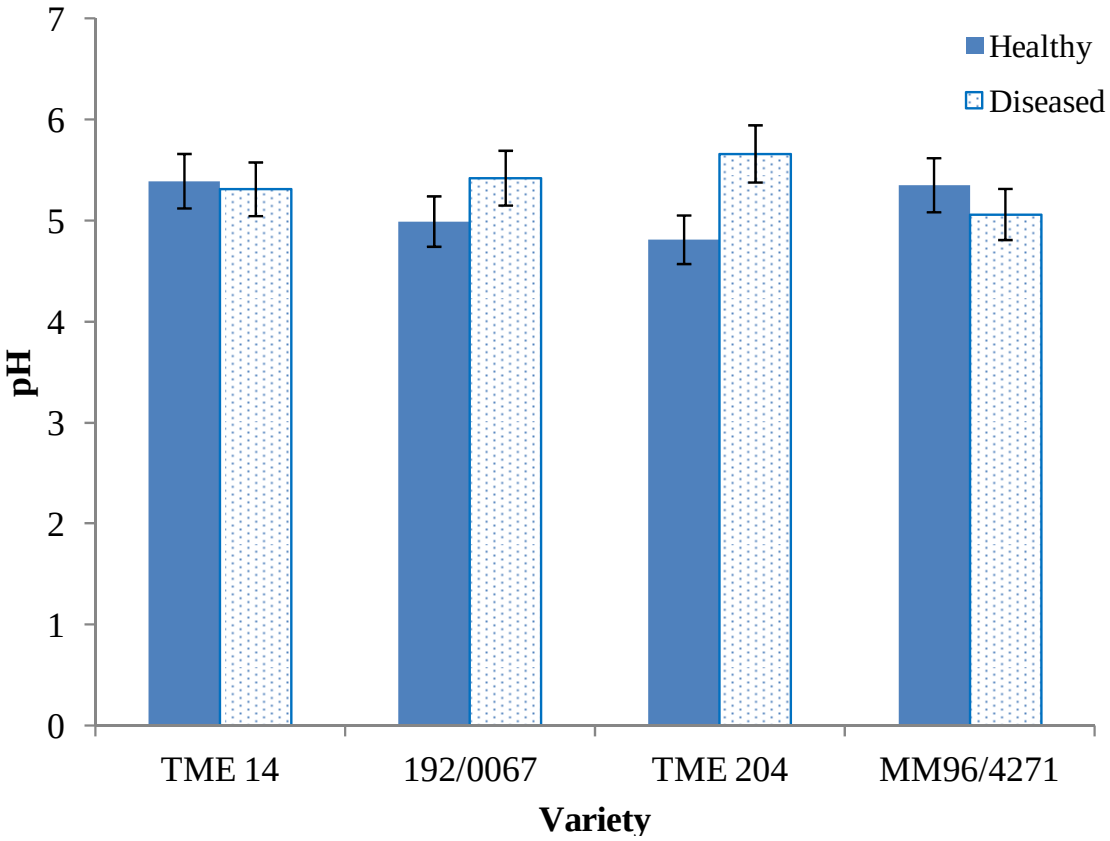

Figure 1. pH of the starches from different varieties from both diseased and healthy plots.

in form of starch.

Much as there were no significant differences among varieties and the treatment groups for pH, a clear pattern was observed among the tolerant and susceptible varieties with starch pH being higher for diseased susceptible plots and lower for diseased tolerant plots (Figure 3). However the reverse was true for the healthy plots with significant differences observed for TME 204 for the diseased and healthy plots. The pH of the starch solution is affected by a number of factors but importantly the chemical composition of starch constituents. In particular it is affected by the amount of soluble material in starch which depending on the composition and charge differences will affect the pH of any starch solution. It also affected positively by the amount of available starch (r= 0.762) although accumu-lation of fibrous material within the root had a negative effect on the pH (r= -0.560). In particular the pH of the starch was also affected by the level of cyanide (r=0.468) in the health treatments although in the diseased treatments changes in protein content also had a significant effect on the pH (r=-0.680).

The starch content measured as the total amount of enzyme digestible starch was low among the healthy plots but high among the diseased plots (Table 1). There were significant differences ($P<0.05$) among the varieties used in each of the treatments for starch content, implying that the amount and type of digestible starch may change with viral infection. This may result into deposition of low molecular weight starch derived oligo saccharides and related compounds (Shaltin and Wolf, 2000) which on digestion produce high sugar contents that are quantified as starch partly explaining the high starch contents observed for the diseased plots. The results also show that starch based accumulations depend on either the tolerance or susceptibility of the plant and how the plant responds to viral attack. This is because different rates of respiration and hence starch degradation occurs in different plants depending on the variety in regard to the requirements of the plant in any particular state (Shaltin and Wolf, 2000).

As expected, the reducing sugar contents were higher for the diseased plots (0.10-0.18mg/g) compared to the healthy plots (0.052-0.071 mg/g) (Table 1). Significant differences ($P<0.05$) occurred between the two treat-ments for all the test varieties and among the varieties themselves. However, the accumulated sugars for the diseased plots were higher in quantity among the susceptible varieties I/92/0067 and TME 204 (0.138-0.178 mg/g) compared to the tolerant varieties TME 14 and MM96/4271 (0.101-0.132 mg/g). In contrast, for the healthy plots, the reducing sugar contents fell within the

Table 1. Composition of starch and reducing sugar content, and total fiber for the different starches.

Variety	Starch content (%)		Reducing sugar content (%)		Fiber (%)	
	H	D	H	D	H	D
TME 14	73.23±0.40	77.34±0.72	9.47±0.018	19.1±0.014	5.04±0.96	7.50±1.36
192/0067	65.33±0.51	78.43±0.11	11.55±0.019	17.8±0.032	4.92±0.70	9.28±1.18
TME 204	75.45±0.24	77.45±0.06	9.54±0.016	18.8±0.068	4.72±0.83	9.84±4.32
MM96/4271	72.92±0.21	77.59±0.09	12.19±0.023	18.2±0.035	4.72±0.70	7.96±3.25
L.S.D	0.02130	0.0789	0.02130	0.05100	1.082	0.879
CV %	32.4	12.4	27.6	27.7	16.6	7.6

H=Healthy sample; D=diseased sample.

Table 2. Protein content and Cyanide levels from the different varieties.

Variety	Protein content (%protein)		Cyanide content (mg/g)	
	H	D	H	D
TME 14	0.53±0.09	0.57±0.09	1.407±0.525	1.615±0.873
192/0067	0.58±0.07	0.54±0.09	0.815±0.388	1.626±0.557
TME 204	0.60±0.03	0.18±0.06	0.892±0.371	1.540±0.744
MM96/4271	0.77±0.15	0.89±0.12	0.981±0.384	1.916±0.946
L.S.D	0.1496	0.1494	0.3461	0.878
CV%	18.0	20.3	25.3	38.7

Mean values of four analyses are presented. H=healthy sample; D=diseased sample.

same range with no apparent significant differences observed among them. It was also observed that reducing sugars accumulated with decrease in starch yield/starch content (Table 2). The accumulation of reducing sugars has been observed in many instances especially where stress (abiotic and biotic) is observed and in particular, in viral stress related effects (Fraser, 1987). This may be due to the compromised photo-synthetic processes by the virus which result into deposition of sugars as important metabolites for viral metabolism (Tecsi et al., 1994) or it may be due to remobilization of starch resources from the sink by the plant (Goodman et al., 1986) which may help the plant to improve its defensive mechanism. Viral infection can also result into altered localization of sugar and other carbohydrate resources leading to their accumulation in the root (Haritatos et al., 1996). Ideally, reducing sugars followed an inverse pattern as for starch, with more percentages increments observed for the susceptible varieties implying that there was possible degradation of starch due to the effects of viral attack.

The fiber content was also significantly different ($P<0.05$) between the healthy and diseased plots with the diseased plots having high fiber contents (7.4-13%) compared to the healthy plots (3.9-6.0%) showing an average 50% increment in fiber content (Table 1). In particular, the tolerant varieties which had lower fiber contents in the healthy state accumulated more than 55% fiber in the diseased state that can be attributed to accumulation of non-starch and other indigestible materials in the storage root. On further analysis, the percentage proportion of starch to fiber was determined for each of the test varieties among the two different treatments used and was found to be low for the diseased plots (51-63%) while it was considerably high for the health plots (74-84%). Similarly, the percentage proportion of starch to reducing sugars was found to be higher in healthy plots than diseased plots pointing to possible starch degradation to sugars in diseased plots.

The protein content was determined as root starch protein percentage using the BSA as a standard. A narrow range for protein content was observed in the healthy plots (0.52-0.77% protein) compared to the diseased plots (0.18-0.91% protein) (Table 2). This implied that different varieties accumulated different amounts of protein in the diseased state compared to the healthy state where protein accumulation was uniform. Significant differences ($p< 0.05$) were observed between the diseased and healthy plots for individual varieties where among the tolerant varieties the diseased plots had higher protein contents compared to the healthy plots (about 10-20% increments) while among the susceptible varieties the diseased plots having lower protein contents (12-58% less). Changes in the amount of available protein may be due to either shutdown of protein synthesizing processes by the viral components (Tecsi et

Table 3. Percentage lignin content (Klason lignin) for milled cassava flour from roots with different CBSD scores.

Variety	Score 5 (% lignin)	Score 4 (%lignin)	Score 3 (%lignin)	Score 2 (%lignin)	Score 1 (%lignin)
TME204	85.32± 0.82	60.77± 0.75	34.98± 0.38	27.01± 0.73	8.47± 0.06
I/92/0067	73.64± 1.77	64.98± 2.02	40.19± 3.12	26.25± 0.50	12.41± 0.51
MM96/4271	81.52± 1.03	55.86± 0.28	40.96± 0.43	20.96± 0.31	9.72± 0.12
TME 14	55.54± 1.29	32.61± 2.34	19.38± 0.80	9.68± 0.27	8.08 ± 0.49

al., 1996) or due to the hijack of protein synthesis by the virus and using it for its advantage (Good man et al., 1986). It may also be due to production of defensive proteins mounted by the plant against the virus (Shaltin and Wolf, 2000).

For the cyanide content, over all increments were observed among the diseased plots regardless the tolerance levels of the varieties used. Much as there were significant differences ($p<0.05$) among the varieties for the cyanide content in the healthy plots, the diseased treatments accumulated cyanide in almost a similar way and thus there were no significant differences among them (Table 3). In particular over 60% increments were observed for the tolerant variety MM96/4271 a known non cyanogenic variety while about 30% increments were observed for the cyanogenic variety TME 14. Overall increase in cyanide content point to the role of this secondary metabolite in plant defense (Fu et al., 2010) much as the increments did not depend on prior cyanide accumulation within a particular variety. Since cyanide is derived from existing carbon sources; its accumulation may explain the losses in starch based metabolites observed as a function of utilized reducing sugars. However, on the dietary and food functionality point, viral attack is risky since it renders the root toxic and hence unpalatable for food or feed (Baguma et al., 2003).

The lignin content was determined depending on the root CBSD score and was found to increase with increase in the CBSD score. In the tested varieties, the lignin content ranged from 55-85% at score five (5), 31-60% at score four (4), 18-35% at score three (3), 8-27% at score two (2) and 7.5-13% for the health tubers (Table 3). Variations within the varieties were also observed with TME 204 having the highest lignin content in all cases except for the health plots. TME 14 had the lowest lignin contents in all cases even for the healthy varieties with significantly low lignin contents even at score 2 and score three. The accumulation of lignin in diseased plants has been reported before in some studies (Morrison et al., 1995), although it has not been reported in cassava. The causes may range from a number of physiological changes resulting from disease causing agents exploiting the phenyl propanoid pathway and genetic manipulation of genes that shut down starch synthesis and promote lignin deposition (Rastogi and Dwivedi, 2008). The high lignin percentages at score five and four may describe the selective accumulation of lignols in the root which increase with root growth time as has been evidenced in the progressive necrotic patches in the roots with growth time (Odpio et al., 2013). Given the high lignin percentages at both score 2 and score 3, it was observed that the necrotic patch size and necrosis intensity do not correlate well with the accumulation of lignin. Such roots with scores between 2 and 3 have already accumulated lignin as evidenced by the changes in the root color from whitish to brownish patches or parts of the root.

The effects of viral attack on the components of starch that is the amylose and amylopectin content were analyzed spectrophotometrically. The results given are absolute absorbance values reflecting the differences in quantities of these starch components after iodine staining. In all cases, the quantity of amylose was high in the healthy state compared to the diseased (Table 4) with major reductions observed for the susceptible varieties (38.5% reduction) compared to the tolerant varieties (29.7%). The same applied to the amylopectin content much as the reductions were not so different when the tolerant varieties were compared to the susceptible varieties. The amylose/amylopectin ratio was similar across all the varieties and lower in the healthy plots compared to the diseased plots, too. This implies that there was selective accumulation of amylose in the diseased plots compared to amylopectin content which may be due to alteration in the starch synthesis pathway but importantly the enzymes involved in the synthesis of starch (Baguma et al., 2003). Such changes in the accumulation of amylose especially in the diseased state can also serve to explain the differences observed for starch solution properties. The relationship between amylose and amylopectin content and protein content in the diseased and healthy state was also tested where in the diseased state; reductions in protein were accompanied with reductions in starch components unlike in the healthy state. Therefore the deposition of lignified and brown materials within the root in the diseased state may be as a result of compromised amylopectin and protein synthesis especially for the susceptible varieties. Further still, significant differences ($p<0.05$) were observed for amylopectin content in the diseased treatments whereas no significant differences were observed for amylopectin in the healthy treatments showing that viral attack has significant effects on the starch components amylose and amylopectin rather than total starch contents as earlier observed.

Table 4. Comparison of Amylose and Amylopectin content for the different variety starches sources.

Variety	Amylose (Abs)		Amylopectin (Abs)		Ratio (Amylose:Amylopectin)	
	H	D	H	D	H	D
TME 14	0.471±0.046	0.299±0.072	0.448±0.046	0.267±0.069	1.05	1.12
192/0067	0.477±0.074	0.263±0.076	0.452±0.076	0.230±0.074	1.05	1.14
TME 204	0.386±0.075	0.262±0.058	0.366±0.076	0.238±0.056	1.05	1.10
MM96/4271	0.413±0.057	0.319±0.070	0.389±0.059	0.287±0.067	1.06	1.11
L.S.D	0.0647	0.0798	0.0657	0.0774	Average ratio: 1.05	Average ratio: 1.12
CV%	11.1	20.8	11.8	22.6		

Mean absorbance of 10 analyses at 620 and 680 nm. H=Healthy sample; D=diseased sample.

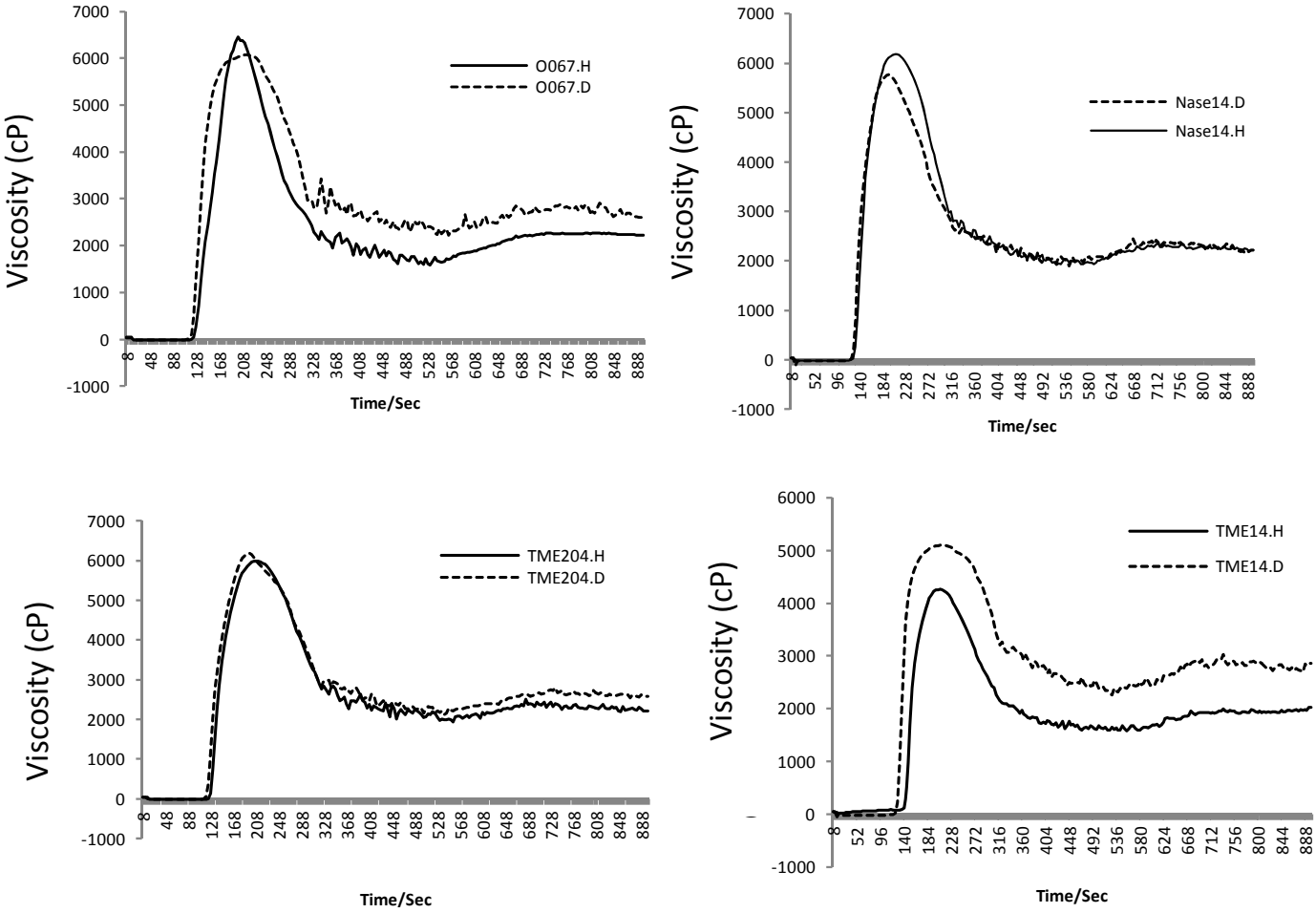

Figure 4. Pasting curves of cassava starch from diseased and healthy plots as compared across the test varieties.

From the pasting curves (Figure 4), differences in the starch pasting properties were observed among the test varieties. High peak viscosity was observed for variety I/92/0067 in both healthy (average 6408 cP) and diseased (average 6114 cP) plots compared to the rest of the test varieties. Low paste viscosity was observed for TME 14 still in both healthy (average 4282 cP) and diseased plots (5110.5 cP). Significant differences ($P<0.05$) were observed for the peak viscosity, break-down viscosity and the final viscosity among the test

varieties although no significant differences were observed for the peak time. The pasting temperatures were also not significantly different much as TME 14 had higher pasting temperatures (71.2°C) compared to other varieties were the pasting temperature ranged from 67-69°C.

A mixed reaction was observed when starch from diseased cassava plots was compared with starch from healthy plots in terms of the peak viscosity where in varieties NASE 14 and I/92/0067 the healthy plots had higher peak viscosity while in varieties TME14 and TME 204; the diseased plots had higher peak viscosity. Significant differences ($P<0.05$) were observed among the diseased and healthy plots for break down viscosity, final viscosity and the pasting temperatures much as no significant differences were observed for setback viscosity, peak time and the trough viscosity. However, significant ($P<0.05$) differences were observed for the peak area which was bigger in the diseased plots compared to the healthy plots signifying changes in the starch pasting properties.

From the above, it can be noted that CBSD has significant effects on the quality properties of the starch produced. In particular, it affects the processing attributes of the starch. Such effects seem to be variety specific giving hope for possibilities of selection of varieties that can be used for various purposes even in the diseased state. However, coupled to effects in starch quantity properties, the detrimental effects of the disease are manifested.

Conclusions

From this study, it was observed that CBSD affects the accumulation of storage root components in addition to altering the composition and molecular structures of these components. Such effects are thought to be linked to altered carbohydrate and nitrogen based compounds metabolism much as at this stage it is not clear whether it is for viral establishment or for plant defensive strategies. However, it is clear that viral attack in the cassava varieties tested has significant and broader effects on the cassava growing communities that use it for food. The inferences in this study show that symptom based selection for susceptibility is key much as broad range selection of tolerance to viral diseases may need to employ biochemical based manifestations in the cassava root in regard to observed leaf based symptomology. In particular, alterations in carbohydrate based metabolite quantities and the quality of starch/changes in starch components is very key in this aspect. It is easy to use and can be employed on a number of samples producing results faster and in a reliable fashion. Nitrogen based metabolites such as proteins and secondary metabolites such as cyanide are also key selection indicators to supplement genetic based selection tools for easy identification of viral tolerant varieties. However, more work needs to be done to understand the interaction of the root based biomass accumulated in form of fiber and starch with the main plant photosynthesizing organs, the leaves. Such will provide lasting solutions and dependable tools for biochemical based selections.

Conflict of interest

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

Our studies were made possible by financial support from the East African Agricultural Productivity project, hosted at the National Crops resources Research Institute (NaCRRI). Special thanks to Phillip Abidrabo and the pathology team at NaCRRI for their valued input and Mrs Genevieve Einau for technical support.

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