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Progression of Cassava Brown Streak Disease (CBSD) in infected cassava roots in Uganda

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Abstract

Cassava Brown Streak disease (CBSD) has and continues to be a major threat to the cassava industry in Uganda. The most economically damaging symptom of CBSD occurs on the roots as a yellow/brown, corky necrosis. However, the onset and development of this necrosis is not known. Therefore, this study was conducted to understand the progression of CBSD root necrosis.The experiment was conducted at Namulonge (central Uganda), where the CBSD pressure and whitefly population is high. Four CBSD susceptible genotypes (TME204, TMSI92/0067, MH97/2961, and Bamunanika) and five CBSD tolerant genotypes (TME14, NASE 3, NASE 1, MM96/0686 and 28-TME 14) were used. The experiment was laid out in a split-plot factorial experiment with three replicates. CBSD root necrosis was assessed at 4 months after planting (MAP) and, thereafter, at monthly intervals until 12 MAP. Results indicated significant differences (P<0.001)among reaction grades (susceptible and tolerant), genotypes and sampling times. CBSD root necrosis commences as early as 4 MAP in susceptible genotypes with a severity of 2 and incidence of 16.67%. These findings have important implications for CBSD breeding particularly when evaluating seedlings and/or clonal plants that often have different number of roots.

Key words: Cassava brown streak virus, disease pressure, root necrosis

Introduction

Cassava brown streak disease (CBSD), which is caused by *Cassava brown streak virus* (*Ipomovirus: Potyviridae*) (Monger *et al*., 2001), affects the yield and quality of cassava storage roots. CBSD is one of the major challenges to optimal cassava productivity in Uganda, and in the whole of the East African region. CBSD produces characteristic symptoms on the leaves, stems and roots of the affected cassava plant (Alicai *et al*., 2007). The economically damaging symptoms occur on the roots as a yellow/brown, corky necrosis in the starch-bearing tissues (Hillocks *et al*., 2001). The necrosis begins as discrete areas, but in highly susceptible varieties, it may affect most of the root parenchyma (Nichols, 1950; Hillocks *et al*., 1996; Hillocks and Jennings, 2003).

Roots of affected plants often show necrosis in the starchy tissues, malformations and constrictions that further decrease their suitability for human consumption. Thus, the disease has two typical effects, reduction of root yield and quality. This in turn affects marketability of the roots (Hillocks *et al*., 1996). Indeed, yield losses of up to 70% associated with CBSD have been reported. The characteristic root symptoms are used as

a measure of resistance/tolerance to CBSD. For example, a score of 1 refers to genotypes whose roots have no necrotic tissues. A maximum score of 5 refers to roots with > 25% necrotic tissue (Gondwe *et al.*, 2003). In practice, these assessments are made at harvest, which are usually not less than 12 MAP. Unlike above-ground CBSD foliar symptoms where several assessments are made on leaves and stems, only one evaluation is usually made on roots at harvest. This limited assessment of disease progression complicates selection and could perhaps explain the limited genetic progress made in CBSD resistance breeding compared to cassava mosaic resistance breeding.

There is limited information on progression of CBSD in the roots. Work conducted in Kenya during the 1970s assessed two varieties and showed that roots of infected plants showed extensive areas of necrosis but exhibited no difference in root weight between infected and symptomless plants (Bock, 1994).

Related observations made during surveys in Tanzania suggested that some plants with severe CBSD symptoms produced smaller roots than symptomless neighboring plants (Hillocks and Raya, Unpublished data). Outstandingly, these studies did not describe the progression of CBSD in the root. They only emphasized the final CBSD severity score at harvest. It has since remained unclear if the progression rate of CBSD root necrosis is similar among susceptible and/or tolerant genotypes. This information is valuable in CBSD breeding.To fill this knowledge gap; this study was conducted to determine the progression of CBSD in the infected roots among selected cassava genotypes classified as either susceptible and tolerant to CBSD. Tolerant genotypes were defined at 12 MAP byCBSD root incidence of less than 10%, whereas susceptible genotypes were defined by CBSD root incidence of greater than 30%.

Materials and methods

This study was conducted at Namulonge located in central Uganda at latitudes (E°032.38007, N°01.31055) and at 1135 meters above sea level. Namulonge is characterised by high CBSD pressure and whitefly populations. Four CBSD susceptible genotypes (TME 204, TMS I92/0067, MH97/2961 and Bamunanika) and five CBSD tolerant genotypes: TME 14, NASE 3, NASE 1, MM96/0686 and 28TME 14 (F1 hybrid of TME 14), were selected for the study to form the two reaction grade groups.

For both categories of genotypes, disease free planting materials were sourced from CBSD-free areas in Northern Uganda, which are also characterized by low whitefly (vector) populations. The cassava genotypes, MH97/2961, TMS I92/0067, NASE 3, NASE 1, 28-TME 14 and MM96/0686 are improved genotypes and/or breeding lines used by the National Cassava Programme. Genotypes TME 14 and TME 204 are landraces introduced from West Africa. All these genotypes are being grown by farmers in different parts of Uganda. The genotype Bamunanika is a local landrace that is commonly grown in central Uganda.

The experiment was laid out in a split-plot factorial experimental design with three replicates. Each genotype was represented by single row plots of fifteen plants. The main plot was composed of CBSD reaction grade (susceptible or tolerant genotype), while the sub-plots were composed of the different genotypes under each reaction grade.

A spreader row of a highly susceptible variety TME 204 was included to augment the CBSD inoculum pressure. The established plants were evaluated for CBSD root severity and incidence at monthly intervals starting from 4 to 12 MAP. CBSD foliar symptoms were scored using a standard five point scoring scale (Gondwe *et al.*, 2003) where 1 = no apparent symptoms, 2 = slight foliar feathery

chlorosis, no stem lesions, 3 = pronounced foliar feathery chlorosis, mild stem lesions, and no die back, 4 = severe foliar feathery chlorosis, severe stem lesions, and no die back, and 5 = defoliation, severe stem lesions and die back. On the other hand, CBSD root symptoms were assessed using a scale of 1-5, where 1 = no apparent necrosis, 2 = less than 5% root necrosis, 3 = 5-10% root necrosis, 4 = 10-25% root necrosis, mild root constriction and 5 = >25% root necrosis with severe root constrictions.

On each sampling occasion, one plant was randomly selected, uprooted and assessed. Availability of virus-free stakes was a challenge and this limited the quantity of planting material. We thus decided to sample one plant per genotype in each replicate with multiple sampling dates (i.e., nine data sets) as opposed to having very few sampling dates with more plants sampled per occasion. Moreover, the test genotypes produce an average of at least five roots per plant, which is reasonable to generate CBSD data suitable for this study.

All harvested root(s) per plant were scored. CBSD root incidence was computed as the proportion of roots with CBSD to the total harvested roots expressed as percentage. Foliar symptoms of the entire plot were also assessed at every sampling occasion. The generated data sets were subjected to statistical analysis using Genstat 13th Edition (Goedhart and Thissen, 2010).

Results

A total of 12 cassava genotypes were assessed for root symptom development during a 12 month growth period at Namulonge; nine sampling dates were undertaken during this growth period. Significant differences ($P<0.001$) were recorded for the reaction grade, genotypes and sampling times (Table 1). CBSD root necrosis severity ranged from 1 to 5, while CBSD root necrosis incidence ranged from 0 to 100% (Figure1A). For the susceptible reaction grade, genotype TMS I92/0067 showed its maximum root severity of 5 at 7 MAP, while other susceptible genotypes, notably TME 204, MH97/2961and Bamunanika showed maximum root severity of 3 at either 6 or 7 MAP (Table 2).

For the tolerant reaction grade, genotype TME 14 had a maximum root incidence of 63%

Table 1. Mean squares for CBSD root necrosis incidence, root necrosis severity and foliar severity as influenced by reaction grade, genotypes, sampling time and their interactions in the Split-plot analysis

Source of variation	d.f	CBSD root incidence	CBSD root severity	CBSD foliar severity
Replicate	2	11.1	0.3	0.1
Reaction grade	1	156666.5*	212.5501**	233.02914**
Genotype	8	1967.9*	2.0064*	9.88325**
Sampling time	8	10663.6**	15.3821**	8.01189**
Sampling time x Reaction grade	8	6097.0**	9.2388**	4.42766**
Sampling time x Genotype	64	774.2*	0.6689**	0.35867**
Residual	136	445.6	0.3228	0.09418
Total	227	176625.9	240.434	255.9161

Reaction Grade = tolerant and susceptible categories, d.f = degrees of freedom
*Indicate significance at 0.05 level and **Indicate significance at 0.01 level

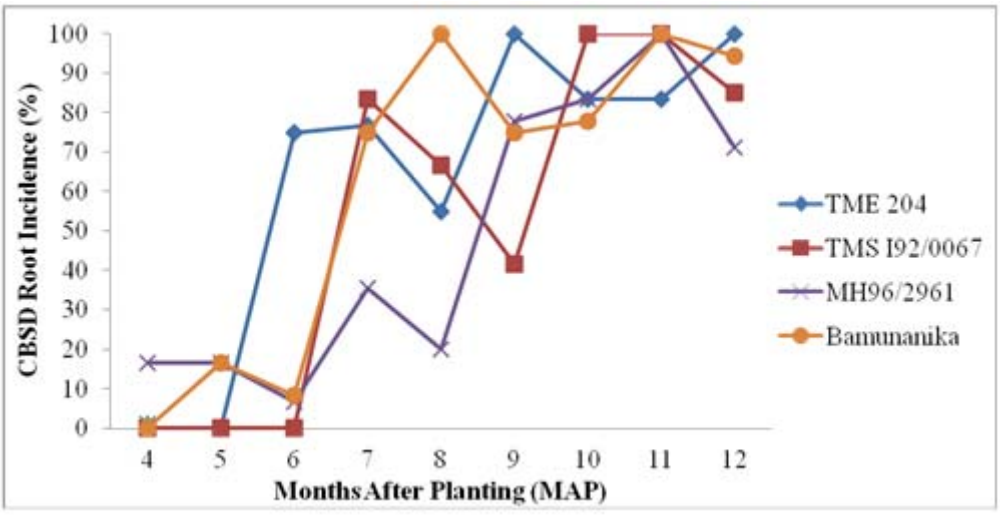

Figure 1A. Progression of CBSD root necrosis in susceptible varieties over a 12 month growth period. Evaluation was based on one random plant harvested at each sampling occasion.

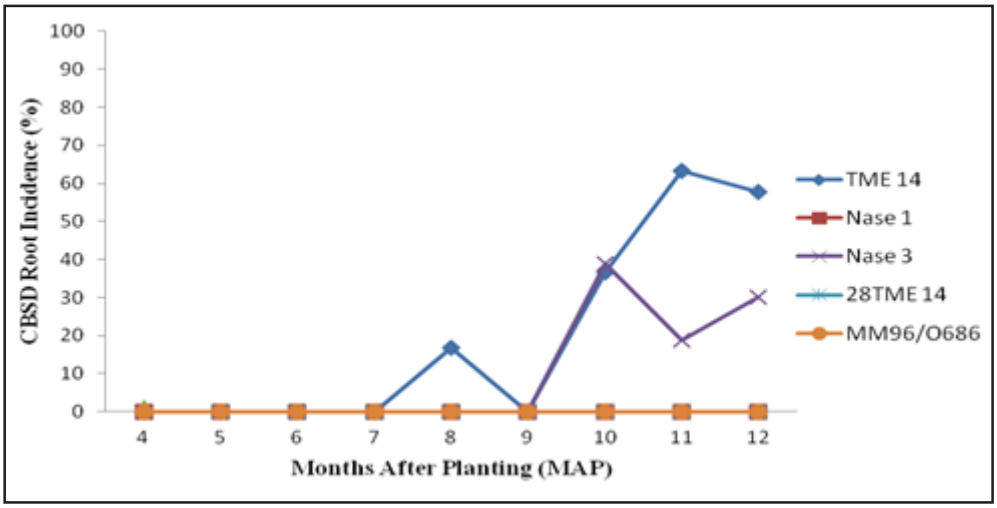

Figure 1B. Progression of CBSD root necrosis in tolerant varieties over a 12 month growth period. Evaluation.

at 11 MAP with a maximum severity of 4 at 10 MAP (Figure 1B and Table 2). With the exception of genotypes TME 14 and NASE 3, other genotypes (NASE 1, MM96/0686 and 28-TME14) had less than 20% root incidence (Figure1B). Genotype 28-TME 14 and MM96/0686 had severity of 1 and CBSD root incidence of zero throughout the evaluation period.

Results further indicated high, positive and significant correlations between CBSD foliar symptom severity and CBSD root necrosis severity ($r = 0.63$, $P<0.001$) for susceptible genotyes. Similarily, there were high, positive and significant correlations between CBSD foliar symptom severity and CBSD root necrosis severity ($r = 0.61$, $P<0.001$) for tolerant genotyes. A linear regression

Table 2. Maximum CBSD root necrosis severity scores for both susceptible and tolerant genotypes across the nine sampling occassions

Reaction grade	Genotypes	Months After Planting (MAP)								
		4	5	6	7	8	9	10	11	12
	TME 204	1	1	3	4	5	4	5	5	5
	TMS I92/0067	1	1	1	5	4	4	4	5	4
Susceptible	MH96/2961	2	2	2	3	4	5	3	4	4
	Bamunanika	1	2	3	3	5	5	5	5	5
	TME 14	1	1	1	1	3	1	4	4	3
	NASE 1	NR	1	NR	NR	NR	1	1	1	1
Tolerant	NASE 3	1	1	1	1	1	1	3	3	3
	28-TME 14	NR	1	1	1	NR	1	1	1	1
	MM96/0686	1	1	1	1	1	1	1	1	1

Scores based on 1-5 scale of Gondwe *et al*, (2003) where 1 = No apparent necrotic tissue, 2 = Less than 5% necrotic tissue, 3 = 5-10% necrotic tissue, 4 = 10-25% of necrotic tissue with mild root constriction and 5 = >25% necrotic tissue and severe root constriction. NR = No roots were found at that sampling time

coefficient between CBSD foliar severity and CBSD root severity was high (R^2 = 0.658). Generally, there was more disease pressure on susceptible than tolerant genotypes (Table 2).

Discussion

The main objective of the study was to describe the progression of CBSD root necrosisin susceptible and tolerant cassava genotypes. This information is important to fine-tune CBSD root evaluation methods and to explore the management potential of early harvest to reduce harvest losses due to root necrosis. The results indicate that CBSD root necrosis progression varies among genotypes. Among the susceptible genotypes, high severity scores of 3 or more are observed as early as six months with incidence of >50%. On the other hand, for the tolerant genotypes, root necrosis severity scores of 2 or 3 are observed at 11 MAP, with incidences of <20%. These findings have two important implications for CBSD breeding.

First, it suggests potential value for apreliminary evaluation of CBSD root symptoms in segregating progeny at six months. This can be done at both seedling and clonal evaluation stages. This type of screening would significantly reduce the number of progeny to advance for further evaluation and possibly increase the efficiency of selection. Cassava breeders often handle thousands of clones and will not have to wait for 12 months in order to ascertain the reaction grade of segregating progeny. Fewer putative resistant clones would be advanced and evaluated further at 12 MAP after a longer period of virus exposure. Importantly, at 6 MAP, reaction to CMD can also be ascertained; making combined CMD and CBSD selection possible. It suffices to note that this can only be possible where CBSD inoculum pressure is high.

Secondly, because CBSD root necrosis progression increases with plant age, it is therefore rational to suggest that, early maturing varieties (that can yield >25t ha^{-1} at 6 MAP) could be another option for CBSD

management.This is true since these early maturing varieties shall have been harvested by the time CBSD root necrosis develops up to the severity score of 3. In fact some cassava farmers resident in CBSD hotspots in Tanzania often adopt earlier harvesting as a CBSD management strategy.

The positive correlation between CBSD foliar symptom severity and root necrosis severity showed there was often simultaneous increase in both types of symptoms. This correlation suggests that in some cases foliar severity can be used to determine the extent of root severity and avoid the need for uprooting the plants for assessment. However, this association is not consistent enough particularly when a large number of genotypes are evaluated. For instance, sometimes genotypes with no foliar CBSD symptoms show high levels of root necrosis and sometimes genotypes with significant CBSD foliar symptoms do not show root necrosis.

The coefficient of determination (R^2) of 65.8 percent means that it is only 65.8% of CBSD foliar severity observed that could have been directly linked to CBSD root necrosis, thus, 34.2% of CBSD root necrosis could not be attributed to the foliar severity observed. This is in agreement with the study conducted by Hillocks *et al.* (1996) where they found 21% of the genotypes with severe foliar CBSD symptom without root necrosis. For CBSD severity, it indicates that different genotypes have different levels of tolerance to CBSD. This difference in tolerance is shown by different critical stages of CBSD root necrosis development. These observations have also been reported in earlier studies conducted in coastal Kenya (Munga, 2008).

In conclusion, this is the first study to examine the progression of CBSD root necrosis symptoms at different months after planting. Though based on a rather limited number of root samples per sampling occasion (owing to challenges in availability of planting material), they provide useful insights on the progression of CBSD in cassava roots. These observations lay a foundation for efforts to fine-tune CBSD evaluation methods. Further studies that associate root necrosis phenotypes with virus titer in the root tissue will increase our understanding of CBSD root necrosis development and, hence, increase CBSD resistance breeding efficiency.

Acknowledgements

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