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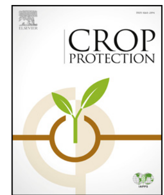
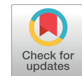


Resistance of advanced cassava breeding clones to infection by major viruses in Uganda



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ABSTRACT

Cassava brown streak disease (CBSD) and cassava mosaic disease (CMD) are two viral diseases that cause severe yield losses in cassava of up to 100%, thereby persistently threatening food and income security in sub-Saharan Africa. For effective management of these diseases, there is a critical need to develop and deploy varieties with dual resistance to CBSD and CMD. In this study, we determined the response of advanced breeding lines to field infection by cassava brown streak viruses (CBSVs) and cassava mosaic begomoviruses (CMBs). This aim helped in identifying superior clones for downstream breeding. In total, 220 cassava clones, three in uniform yield trials (UYTs) and 217 in a crossing block trial (CBT), were evaluated for virus and disease resistance. Field data were collected on disease incidence and severity. To detect and quantify CBSVs, 448 and 128 leaf samples from CBSD symptomatic and symptomless plants were analyzed by reverse transcription PCR and real-time quantitative PCR, respectively. In addition, 93 leaf samples from CMD symptomatic plants in the CBT were analyzed by conventional PCR using CMB species-specific primers. In the CBT, 124 (57%) cassava clones did not express CMD symptoms. Of the affected plants, 44 (55%) had single *African cassava mosaic virus* infection. Single *Cassava brown streak virus* (CBSV) infections were more prevalent (81.6%) in CBT clones than single *Ugandan cassava brown streak virus* (UCBSV) infection (3.2%). Of the three advanced clones in the UYT, NAROCASS 1 and NAROCASS 2 had significantly lower ($P < 0.05$) CBSD severity, incidence, and CBSV load than MH04/0300. In the UYT, only 22% of samples tested had CBSVs, and all showed a negative result for CMBs. The low disease incidence, severity, and viral load associated with NAROCASS 1 and NAROCASS 2 is evidence of their tolerance to both CBSD and CMD. Therefore, these two cassava clones should be utilized in CBSD and CMD management in Uganda, including their utilization as progenitors in further virus resistance breeding.

1. Introduction

Cassava brown streak disease (CBSD) and cassava mosaic disease (CMD) have persisted as major constraints to the production of cassava (*Manihot esculenta* Crantz) in sub-Saharan Africa (SSA), where the crop serves as a major staple food (Alicai et al., 2007; Mohammed, 2012). Storage roots of plants affected by CBSD have a brown necrotic rot and are unfit for consumption. Furthermore, the starch content of CBSD-affected storage roots is greatly reduced and of inferior quality (Nuwamanya et al., 2015). By contrast, storage roots of plants severely affected by CMD fail to bulk because their leaves become chlorotic and mottled, thus having impeded photosynthesis and leading to stunted growth (Mohammed, 2012). Both viral diseases are perpetuated from one season to another through the practice of farmers using stem cuttings from diseased plants as planting material (Hillocks and Jennings, 2003). Farmers in Uganda and Africa generally obtain cassava planting materials from stems of their previous crop or neighbors' fields. The virus status of the planting materials is often unknown because symptoms may not be apparent or leaves may have withered and dropped after crop harvest. This practice leads to the spread of viral diseases to new fields and often advances into disease epidemics. This commonly results in yield losses of up to 100% due to CBSD and CMD, either singly or in combination, thus threatening food security in the region (Hillocks, 2003). In addition, the year-round occurrence of high whitefly (*Bemisia tabaci*) vector populations in many parts of eastern Africa escalates the spread of CMD and CBSD.

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CBSD is caused by two single-stranded RNA viruses of the genus *Ipomovirus*, *Cassava brown streak virus* (CBSV) and *Ugandan cassava brown streak virus* (UCBSV), often jointly referred to as CBSVs. Both viruses belong to the family Potyviridae (Mbanzibwa et al., 2009; Winter et al., 2010). The most prevalent viruses that are causal agents of CMD in SSA are single-stranded DNA bipartite cassava mosaic begomoviruses (CMBs), *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (EACMV), of the family Geminiviridae (Bock and Woods, 1983; Swanson and Harrison, 1994). All these viruses are transmitted by *B. tabaci* (Maruthi et al., 2005), and the disease is spread through the use of infected planting materials. The high demand for cassava planting materials and the lack of functional seed systems for the crop have resulted in the movement of uncertified stem cuttings to be used as planting materials across communities, thereby increasing the spread of the viral diseases. Unless CBSD is controlled through appropriate management strategies, it could potentially become a menace across SSA, including West Africa where the major cassava-producing countries are located.

Effective management of CBSD and CMD will heavily rely on the deployment of resistant varieties to farmers. Fortunately, concerted efforts on the genetic improvement of cassava in Uganda have generated large breeding populations with several elite clones deemed as either tolerant or resistant to both CBSD and CMD (Kawuki et al., 2016). Importantly, genotypes with durable dual resistance to CBSD and CMD should be a target for breeders when screening clones for variety selection. Thus, it is imperative that breeding clones are selected for advancement considering resistance to virus signified by the viral load in plant tissues and the response of genotypes to the diseases under field conditions. Therefore, in this study, we determined resistance or tolerance to infection by CBSVs and CMBs (and resulting CBSD and CMD) in a panel of elite breeding clones, and this is critical for facilitating the development of resistant cassava varieties.

2. Materials and methods

2.1. Test materials and study design

The test materials evaluated in this study include elite clones under evaluation in uniform yield trials (UYTs) and the breeding lines in the crossing block trial (CBT) conducted from 2014 to 2015. The CBT was performed at Namulonge, Wakiso district (Central Uganda), and UYTs were performed at the four major cassava-producing districts: Arua (north-western), Kamuli (eastern), Kaberamaido (north-eastern), and Wakiso. Each of the test sites was located in unique agro ecology, thus differing in cassava viral disease pressure, vector population, and major climatic parameters including mean annual temperature and rainfall. Kaberamaido is located at an elevation of 1080 m.a.s.l., with an annual average temperature of 23.7 °C and annual average precipitation of 1302 mm. Kamuli has an elevation of 1100 m.a.s.l., with an annual average temperature of 21.6 °C and annual average precipitation of 968 mm. Arua is at an elevation of 1215 m.a.s.l. and is characterized by an annual average temperature of 22.9 °C and annual precipitation of 1404 mm. Wakiso has an elevation of 1200 m.a.s.l., with an annual average temperature of 21.8 °C and annual average precipitation of 1377 mm.

Three clones (NAROCASS 1, NAROCASS 2, and MH04/0300) were evaluated in the UYTs for resistance to CBSD and CMD and adaptation to the locations. The officially released CBSD-tolerant variety NASE 14 was included as a control. The NAROCASS 1 clone bred as NAM 130 in Uganda was a selection made from open-pollinated seeds introduced from Tanzania. The NAROCASS 2 clone whose pedigree is MM2006/0130 was bred as MM06/0130 in Uganda. NASE 14, a genotype of pedigree MM96/4271 and bred as MM192/0248, is an IITA introduction officially released in Uganda. Experimental plot sizes were 6 m × 6 m, laid out in a randomized complete block design with four replications per site and plant spacing of 1 m × 1 m. In the CBT, a total of 217 cassava breeding lines, which were part of NaCRRI cassava training population being used in genomic selection, were planted as single-row plots of 10 plants and monitored for disease severity and incidence. To increase the amount of CBSV inoculum, infector rows of the CBSD-susceptible variety TME 204 were planted between test plots. Disease incidence and severity data were collected at 3, 6, and 12 months after planting (MAP).

2.2. Field assessment of CBSD and CMD

The CBSD and CMD severities were assessed and recorded for each cassava plant in the trials at 5, 8, and 11 MAP. CBSD symptom severity was scored on a scale with a rating from 1 to 5 points: 1 = no apparent symptoms; 2 = slight foliar feathery chlorosis and no stem lesions; 3 = pronounced foliar feathery chlorosis, mild stem lesions, and no dieback; 4 = severe foliar feathery chlorosis, severe stem lesions, and no dieback; and 5 = defoliation, severe stem lesions, and dieback (Gondwe et al., 2003). The CMD was scored on a scale of with a rating from 1 to 5 points: 1 = no symptoms observed (shoot healthy); 2 = mild chlorotic pattern on most leaves, mild distortions at the bases of most leaves, with the remaining parts of the leaves and leaflets appearing green and healthy; 3 = a pronounced mosaic pattern on most leaves, with narrowing and distortion of the lower one-third of most leaves; 4 = severe mosaic distortion of two-thirds of most leaves, with general reduction in leaf size and some stunting of shoots; and 5 = very severe mosaic symptoms on all leaves, with distortion, twisting, and severe reduction in leaf size in most leaves, accompanied by severe stunting of plants (IITA, 1990). The CBSD and CMD incidence data were obtained from the number of plants showing foliar disease symptoms, expressed as a percentage of the total number of plants assessed.

2.3. Determination of CBSD root severity, harvest index, and yield in the UYT

The CBSD root severity was assessed by making five cross-sectional cuts with a knife on all the roots and scoring necrosis for each cut section using a pictorial severity scale (rated with 1–5 points) as described by Hillocks and Thresh (2000). The harvested roots together with the ground biomass above were weighed separately, and the weights were used to compute the harvest index (HI) as the ratio of weight of the roots to the total biomass. Yield was estimated using the formula.

Root yield (t/ha) = [root weight (kg/m^2) × 10000]/1000 (Kamau et al., 2011).

Analysis of variance was performed with the mean values of CBSD root incidence, severity, HI, and yield of cassava clones in the UYT to assess whether significant differences existed among the test clones.

2.4. Sample collection for laboratory detection of CBSVs and CMBs

For detection of CBSVs, 448 leaf samples (88 symptomatic and 360 non-symptomatic) were collected from the UYT plots. Non-senescing leaves in the middle to bottom canopy section on a shoot representative of the plant stand were collected. Additionally, a total of 93 CMD-symptomatic leaf samples were collected from the CBT for detection of CMBs. Each sample was placed in a 1.5-ml microfuge tube containing 70% ethanol and stored at room temperature until DNA extraction.

2.5. RNA extraction and RT-PCR for CBSV detection

RNA was extracted from each leaf sample by a modified cetyl trimethyl ammonium bromide (CTAB) method described by Chang et al. (1993). The resultant RNA pellets were dried at room temperature and then resuspended in 50 μl of autoclaved nuclease-free water and stored at −80 °C until analysis. cDNA was synthesized from each of the RNA extracts using SuperScript™ III Reverse Transcriptase kit (Invitrogen,

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