Transmission of *Cassava brown streak virus* by whiteflies

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INTRODUCTION

Cassava is the staple food for >10 million people in Africa (Fig. 1). Cassava brown streak disease (CBSD) is the second most important constraint, after cassava mosaic disease, that affects cassava production in Africa. CBSD is most important in the coastal districts of Kenya, Tanzania, Mozambique and in the lake shore area of Malawi (Fig. 2).

Figure 1. Villagers processing cassava in Tanzania

Figure 2. Distribution of CBSD at altitudes <1000 msl (yellow circle) in the countries (orange coloured) affected in eastern Africa

Typical CBSD symptoms include various patterns of foliar chlorosis especially on older leaves and purple brown lesions on green stems (Fig. 3).

Figure 3. Chlorosis on leaves and stem necrosis

Major yield losses (up to 70%) occur in the form of die back of plants and brown corky necrosis of tuberous roots (Fig. 4), which makes them inedible.

Figure 4. Die back of plants and necrosis of tuberous roots

CBSD is a poorly studied disease and was only recently shown to be caused by *Cassava brown streak virus* (CBSV), a member of the genus *Ipomovirus* of the family *Potyviridae*. The vector of CBSV was unknown, although, the whitefly species, *Bemisia tabaci* (Fig. 5) and/or *Bemisia afer,* are potential candidates because the spread of CBSD coincided with high whitefly populations in field conditions and some ipomoviruses are transmitted by whiteflies.

Figure 5. *Bemisia tabaci* adults, eggs and nymphal stages on a cassava leaf

MATERIALS AND METHODS

Materials and Methods

visiting cassava fields by direct counting on the top and bottom most five leaves. CBSV transmission experiments were initiated by allowing *B. tabaci* and *B. afer* adults to feed on diseased plants for 48 h under laboratory conditions.

About 15-20 insects of each species were then transferred into the clip cages, which were attached to the apex of 10 eight-weeks-old virus-free tissue-cultured cassava plants (var. Albert) for the transmission of CBSV (Fig. 6). The virus was inoculated four times to the same 10 target plants in two weeks time period.

48 h acquisition access period on diseased plant

4 inoculations of ~15-20 *B. afer* and *B. tabaci* each

48 h inoculation feeding period on healthy plant

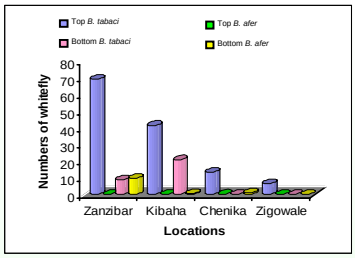
Figure 6. CBSV transmission methodology using both *B. afer* and *B. tabaci* populations

Transmission experiments were carried out using field-collected populations of *B. afer, B. tabaci*, beetles (*Cybocephulus* spp.), thrips and spiralling whitefly. In addition, soil and seed transmission experiments were carried out by using soil from CBSD-affected and -unaffected cassava fields, and seeds from diseased and healthy plants. Total RNA was extracted from whiteflies for the detection of CBSV in them.

RESULTS AND CONCLUSIONS

B. tabaci were most numerous on cassava plants followed by *B. afer* (Fig. 7). >90% of *B. afer* were seen on older leaves where CBSD symptoms were most obvious.

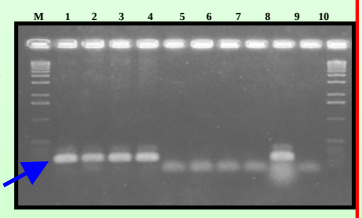
Figure 7. Population of *B. tabaci* and *B. afer* on the top and bottom leaves of cassava plant



Field-collected *B. tabaci*, *B. afer* and other insects did not transmit CBSV. Plants of soil and seed transmission experiments produced no CBSD symptoms six months after planting. Adults of *B. afer* and *B. tabaci* feeding on diseased plants for 48 h had no detectable CBSV by RT-PCR.

Five plants (50%) inoculated using both *B. tabaci* and *B. afer* produced typical CBSV symptoms under laboratory conditions. Infection of CBSV was confirmed by the reverse transcription polymerase chain reaction (RT-PCR) (Fig. 8) using virus-specific primers (Monger *et al*., 2001, *Plant Pathology* 50, 768-775).

Figure 8. Gel electrophoresis photograph of RT-PCR products from infected cassava plants (lanes 1-4), *B. afer* and *B. tabaci* (lanes 5-8), +ve control (lane 9) and water control (lane 10). M = 1 Kb marker.



This is the first conclusive evidence for the vector transmission of CBSV by whiteflies. Further experiments are initiated to investigate if both the whitefly species are required for transmission and to study virus-vector relationships.

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